Functional Foods and Nutraceuticals

Bioactive Compounds

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PREFACE

Nutrition plays an important role in maintaining a healthy life and preventing diseases. Healthy nutrition is among the most important factors in human health in all aspects. The concepts of healthy nutrition and healthy life have gained importance in recent years. Research on healthy living has shown that proper nutrition and physical activity habits play an important role in preventing many health problems, especially obesity. Due to the increase in health problems due to unhealthy nutrition, the concept of 'functional food', which is a new approach in the field of nutrition, has emerged. The special effective bioactive substances in functional foods reduce the risk of disease development and ensure a healthy life. For people to lead a healthy life, it is important to determine which bioactive substance(s) are in the foods and the benefits that these bioactive substances can provide. For this reason, this book is informative about the bioactive compounds of functional foods and nutraceuticals and their effects on health. This book contains the latest updated information and research on functional food and nutraceuticals. The authors of the book chapters consist of academicians who follow the latest literature and scientific changes closely. I would like to thank all the authors of the chapters for their contributions and offer my love and respect. We hope that the book will be useful to all our colleagues and students working in the fields of Medicine, Pharmacy, Veterinary Medicine, Health Sciences, Biological Sciences, Nutrition and Dietetics.

Assoc. Prof. Dr. Haci Ahmet DEVECI
Editor

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OVERVIEW OF FUNCTIONAL FOODS AND NUTRACEUTICALS

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1. Introduction

In the twenty-first century, with the rapid increase in the world population, more efficient use of limited nutrients has become inevitable. In line with the developments in nutrition and medical science, it has become possible to effectively manage the life process of the individual and to increase the quality of life. As a result of these developments, people have become more sensitive and conscious about the qualities of the foods they consume and their contribution to health (1). Today, to lead a healthy life, people are turning to extra beneficial foods that contribute to health, in addition to their normal diet. On the other hand, some problems arise in adjusting the energy balance as a result of individuals needing a less physical activity to reach food and fast-food style eating habits. As a result, the incidence of chronic and difficult-to-diagnose diseases, cancer, diabetes, blood pressure, and cardiovascular diseases along with obesity has increased (2). One of the risk factors for chronic diseases in developed countries is unhealthy eating habits. However, antioxidant-rich foods may have a protective effect against cancer, diabetes, Alzheimer's, Parkinson's, multiple sclerosis, cardiovascular diseases, and other chronic diseases caused by oxidant

molecules (3, 4). With the scientific demonstration of the effectiveness of some food components in the treatment and prevention of diseases, the importance of nutritional support in protecting our health has increased. Depending on these indicators, functional foods and nutraceuticals have become more consumed by the society (5).

2.   Functional Foods

Functional foods meet the body's basic nutritional requirements. In addition, it is also defined as a food or food component containing bioactive substances that provide additional benefits to human physiology and biochemical functions, play a role in the prevention of diseases, and improve health (6, 7). Functional food can be an active ingredient in the natural form of the nutrient, or it can be nutrient-enriched with bioactive elements through technological processes, thus having positive effects on health. All foods that provide the energy and nutrients necessary for life can be defined as functional foods (7-9).

For a food to be defined as a functional food, it must have certain characteristics (5, 10-13).

a)   Functional food should be in the form of food and a part of the daily diet.
b)   Functional food should contain a substance that affects improving body functions or reduces the risk of disease.
c)   Components in functional food should not contain elements that may threaten health.
d)   The nutritional and positive effects of functional food on health should be based on solid foundations in terms of nutrition science and medicine.
e)   Functional food should be comparable to its counterparts.
f)   The quantitative and qualitative characteristics of the food component in the functional food should be determined.
g)   If the functional food has gained a functional quality by undergoing any process, there should not be a decrease in its nutritive value.
h)   Functional food should never be in the form of drugs, capsules, tablets, or any other food supplement.

2.1. Sources of Functional Foods and Their Effects on Health

Functional foods and their bioactive components are extensively studied for improving human health and reducing the risk of chronic disease. Increasing

scientific evidence shows that some food and food components have various biological activities in the human body. The beneficial effects of functional foods on health are due to the bioactive compounds they contain (14-17). Bioactive components, sources, and health benefits of some functional foods are shown in **Table 1** (14-17). Functional foods are divided into two as plant-based and animal-derived functional foods according to their sources (1, 5, 7).

2.1.1. Plant-Based Functional Foods

Plant-based functional foods are also called phytochemicals. Phytochemicals are found in fruits, vegetables, and grains. They are substances that can be taken in the daily diet and protect the human metabolism against various diseases (7, 18). There are approximately 8000 different phytochemical compounds in vegetables, fruits, and grains consumed with diet (7, 19). These compounds; are flavonoids, carotenoids, polyphenols, isoflavones, indoles, lignans, saponins, organosulfur compounds, and monoterpenes. These compounds show immunomodulatory, neuroprotective, anticarcinogenic, antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, antiallergic, and antihypertensive effects. Especially in recent years, it is recommended to consume antioxidant phytochemicals to prevent oxidative stress, which is effective in the development of many chronic diseases (7, 19, 20). Phytochemicals exert their beneficial effects on health in various ways (5, 21).

a) A substrate in biochemical reactions
b) Cofactor or inhibitor in enzymatic reactions
c) Ligands that agonize or antagonize cell receptors
d) Increasing the absorption of essential nutrients
e) Capturing reactive toxic agent
f) Absorbent/sequestrants that bind and remove harmful substances in the intestines
g) Fermenting substrates for beneficial bacteria
h) Those that increase the number of beneficial gastrointestinal bacteria and reduce harmful bacteria

Table 1. Bioactive components, sources,
and health benefits of some functional foods.

BIOACTIVE COMPONENT	SOURCES	HEALTH BENEFITS
Carotenoids and lycopene		
Alpha-carotene	Carrots, pumpkins, sweet,	Neutralizes free radicals
Beta-carotene	potato, spinach, tomatoes	
Lutein, Zeaxanthin	Spinach, carrots, corn,	Supports maintenance
	eggs, citrus, broccoli	of eye health
Lycopene	Watermelon, tomatoes	Supports maintenance
	and tomato products,	of prostate health
	red/pink grapefruit	
Fibers		
Soluble dietary fiber	Oats, barley, beans,	Reduces risk of
Insoluble dietary fiber,	apples, psyllium seed	coronary heart disease
Soluble Fibre	husk, citrus whole wheat,	
Fatty Acids		
Monounsaturated fatty acids	Tree nuts, olive, and	Reduces risk of
	canola oil	coronary heart disease
Polyunsaturated fatty acids		
Omega-3 fatty acids	Walnuts, flaxseeds, tuna,	Supports maintenance
Alpha-linoleic acid	salmon, other fish and oils	of heart, eye, and
Docosahexaenoic acid		mental health
Eicosapentaenoic acid		
Conjugated linoleic acid	Cheese, lamb, turkey, beef	Reduces risk of cancers
Phenolics		
Anthocyanidins, Catechins,	Berries, cherries, grapes,	Neutralizes free
Flavanones, Flavones,	apples, cinnamon,	radicals. Reduces the
Lignans, Tannins	peanuts, tea, flax, citrus,	risk of cancer.
	rye	
Plant Stanols/Sterols		
Stanols/Sterols and esters	Corn, soy, wheat, wood	Reduces risk of
	oils, fortified foods, and	coronary heart disease
	beverages	
Soy Phytoestrogens		Supports bone health,
Isoflavones: Daidzein,	Soybeans, soy-based foods	healthy brain function,
Genistein		and immune system.
Prebiotics and Probiotics		
Inulin, Polydextrose	Grains, onions, garlic,	Supports digestive
Fructooligosaccharides	fruits, honey, fortified	system health and
(Prebiotics)	foods and beverages	calcium absorption
Yeast, Bifidobacteria	Yogurt, dairy products	Supports digestive and
Lactobacilli, and other		immune system.
beneficial bacteria		
(Probiotics)		
Isothiocyanates		
Sulforaphane	Cauliflower, broccoli, kale,	Supports the antioxidant
	broccoli sprouts, cabbage,	defense system by playing
		a role in detoxification.

Phytochemicals show their antioxidant effect by activating enzyme production for the suppression of oxidants and reactive oxygen species. Antioxidants should be taken from natural herbal sources such as vegetables and fruits instead of drugs as much as possible. When antioxidants are taken in balance with various vegetables and fruits, they do not reach toxic levels in the body. Thus, other effective components help to increase the functional effect with a synergistic effect (5, 7, 22, 23).

The Mediterranean diet is nutritional model rich in phytochemicals. In the Mediterranean diet, a daily intake of around 290 phytochemicals is provided by consuming foods with high phytochemical content such as olive oil, legumes, nuts, wine, fruit, and vegetables (7, 24). Fermented vegetables, fruits, and grains are also considered functional foods because they have many bioactive components. Traditional herbal fermented foods include tarhana, pickles, soy products, turnip, table olives, wine, and beer (7, 8, 25).

2.1.2. Animal-Based Functional Foods

Red meat and meat products, seafood and fermented milk products are in the functional food class of animal origin. Red meat is considered a functional food because it contains nutrients such as L-carnitine, taurine, creatine and conjugated linoleic acid (CLA). CLA, which is abundant in meat and dairy products of ruminant animals, has antiatherogenic and anticarcinogenic effects (5, 7, 14, 19).

One of the functional foods of animal origin is fermented milk products. Fermented dairy products are considered functional foods because they contain lactic acid bacteria, bioactive compounds, and metabolites. Studies have shown that fermented milk products have hypocholesterolemic, hypotensive, antioxidant, antimicrobial, antiatherogenic, anticarcinogenic, and antiallergenic effects (7, 26-28). Kefir, kumiss, yogurt, some types of cheese, and other fermented milk products are considered functional foods because they contain bioactive peptides. It is reported that their regular consumption increases the number of *Bifidobacterium* and *lactic acid bacteria*, and decreases the number of *Enterobacteria* and *Clostridia* (7, 26, 29).

Seafood is functional food rich in antioxidants, omega-3 fatty acids, and other bioactive compounds. Omega-3 fatty acids found in fish such as tuna, salmon, mackerel, and sardines are one of the most important functional foods of animal origin. Studies show that omega-3 fatty acids strengthen the immune system, reduce the risk of cancer, diabetes, and cardiovascular disease,

slow down age-related cognitive decline, and alleviate the clinical symptoms of dermatological, allergic, psychological, and neurological diseases (5, 7, 30, 31). Marine algae and oily fish have antiatherogenic, anti-inflammatory, antihypertensive, antioxidant, antiviral, and anticarcinogenic effects due to their high content of docosahexaenoic acid (DHA) and polysaccharides (7, 14, 19).

3.   Nutraceuticals

The term 'nutraceutical' is a hybrid term, consisting of a combination of the word's nutrition and pharmaceutical. Nutraceuticals are products that are isolated or purified from food and sold in medicinal forms proven to have physiological benefits and protect against chronic diseases (16, 32, 33). Nutraceuticals are offered for consumption in the form of drops, capsules, tablets, or different dosages containing extracts or concentrated combinations of bioactive substances in foods. However, for a substance to be accepted as a nutraceutical, it must have no toxic effects and its beneficial effects on health in the treatment and prevention of diseases must be scientifically proven (9, 34-37).

Both functional foods and nutraceuticals contain bioactive substances with the same physiological effect to lead a healthier life. The difference between nutraceuticals and functional foods is the way they are administered. At the same time, the biological and biochemical functions of both are the same (16, 38). The terms nutraceutical and functional food are often used interchangeably. However, the meanings of these terms are different. The conceptual affinity between the terms nutraceutical and functional food may confuse, but it will be easier to explain with an example. For example; Broccoli, carrots, and tomatoes are considered functional foods because they are rich in physiologically active components such as sulforaphane, beta carotene, and lycopene, respectively. In addition, these components are considered nutraceuticals when presented in the concentrated form (capsule, tablet, etc.) (4, 39).

3.1. Sources and Health Effects of Nutraceuticals

The importance of nutrition on health status has been known since ancient times. Bioactive components in foods have positive effects on human health. In recent years, technologies have been developed for the purification and characterization of these bioactive components, which are responsible for the effectiveness of foods in promoting health and preventing disease. The content of bioactive components in natural plants is critical to the production processes

of nutraceuticals. Extensive experimental animal studies, including subacute, acute, chronic, and subchronic toxicity analyses, and clinical studies in humans are required to determine the efficacy and safety of a nutraceutical product (40-42). In recent years, nutraceuticals have had an important share in the health sector in treating and preventing diseases with the advantage of their beneficial effects on health. The beneficial effects of nutraceuticals are seen in the form of a healthy and quality life, a strong immune system, delaying the aging process, prevention and treatment of neurodegenerative diseases, cardiovascular diseases, inflammatory diseases, various types of cancer, diabetes, and similar diseases (42-44).

The main source of nutraceuticals are herbal (ascorbic acid, allicin, α-tocopherol, beta-carotene, beta-glucan, fatty acids, gallic acid, capsaicin, **ısoflavones**, luteolin, lycopene, minerals, phenolic acids, phytosterols, quercetin), animal products (coenzyme Q10, choline, chondroitin, conjugated linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, glucosamine, L-carnitine, lecithin, omega-3 fish oil, sphingolipids, minerals) and microorganisms (*Bifidobacterium bifidum, B.infantis, B.longum, Lactobacillus acidophilus*, *Streptococcus salvarius* (subs. *Thermophilus*), *Saccharomyces boulardii*) (45-47).

Important factors such as rapid developments in science and technology, increasing health care costs, and healthy living through nutrition increase the interest in nutraceuticals day by day. In recent years, the nutraceutical market has become a competitive market for large food and pharmaceutical companies, and many food companies have established nutraceutical divisions with a diverse product range (32, 41). Phytochemicals with nutraceutical importance, their sources, and health benefits are given in **Table 2** (16, 32, 41, 48, 49).

Table 2. Phytochemicals of nutraceutical importance,
their sources, and health benefits

Phytochemicals	Sources	Health Benefits
Allicin	Garlic, onion	Antioxidant, anticancer, hepatoprotective, neuroprotective
Anthocyanins	Blueberries, blackberries black raspberries	Antioxidant, anticancer. Improves brain cognitive performance.
Apigenin	Apple, artichoke, cherry, grapes, nuts, parsley	Antioxidant, anticancer antiinflammatory, antispasmodic,
Caffeic acid	Apple, pears, citrus, vegetables	Antioxidant, anticancer.
Carotenes (α and β)	Carrots, various fruits, and vegetables	Antioxidant, anticancer.
Cathesins	Tea, wine, fruit	Antioxidant, anticancer
Chlorojenic acid	Blueberries, tomatoes, grapes	Antioxidant, anticancer
Cinnamic acid	Cinnamon, balsam tree resins	Antibacterial, antifungal
Curcumin	Turmeric root	Antioxidant, anticancer, antiinflammatory
Ellagic acid	Blackberries,pomegranates, raspberries, strawberries,	An antioxidant and prevents colon cancer
Ferrulic acid	Apple, pears, citrus peanut,	Antioxidant, anticancer.
Gallic acid	Tea, mango, strawberries, soy	Antioxidant, anticancer, antiinflammatory, antidiabetic
Hesperetin	Citrus fruit	Antioxidant anticancer
Lutein	Avocado, corn, egg yolk, green vegetables	Anticancer, reduces the risk of cataract and macular degeneration.
Lycopene	Tomatoes, guava, watermelon	Antioxidant, anticancer.
Naringin	Grapefruit	Antioxidant and reduces cholesterol
Piperine	Pepper	Analgesic, hepatoprotective
Quercetin	Citrus, apples, onions, parsley, tea, red wine	Antioxidant, anticancer, antiallergic, anti-inflammatory
Resveratrol	Berries, grapes, peanuts	Anticancer, antimicrobial, antiaging, antidiabetic
Rutin	Asparagus, buckwheat, and citrus fruits	Strengthens capillary walls
Silymarin	Milk thistle (Silybum marianum)	Antioxidant, anticancer, hepatoprotective
Stigmasterol	Soybean	Anticancer, hypolipidemic, prevention of osteoporosis
Sulforaphane	Cauliflower, broccoli, kale, cabbage, radish,	Antioxidant, anticancer. Supports the antioxidant defense
Ursolic acid	Apple, cranberry, lavender, oregano, rosemary	Antioxidant, antiinflammatory, anticancer, antimicrobial,
Zingiberene	Ginger	Antibacterial, antifungal, carminative

Important factors such as rapid developments in science and technology, increasing health care costs, and healthy living through nutrition increase the interest in nutraceuticals day by day. In recent years, the nutraceutical market has become a competitive market for large food and pharmaceutical companies, and many food companies have established nutraceutical divisions with a diverse product range. Similarly, some pharmaceutical companies have made a rapid entry into the nutraceutical market by purchasing food supplement manufacturers (32, 41).

Today, many products in the nutraceutical category such as fortified cereals, antioxidant vitamins, mineral supplements, cholesterol-lowering foods and tablets, amino acid, and protein supplements have taken their place in the nutraceutical market (32, 48-50). Some commercially available nutraceuticals, their components, and categories are shown in **Table 3** (32, 48-50).

Table 3. Some commercially available nutraceuticals and categories

Marketed Nutraceuticals	Constituents	Categories
Green Tea Max™	Active antioxidants such as green tea, grape seed, and pine bark extract,	Antioxidant and nutritional supplement
Omega woman	Antioxidants, phytochemicals vitamins	Antioxidant and immune supplement
Nutrilite Iron-Folic	Iron and folic acid	Nutritional supplement
Weight smart™	Vitamins and trace elements	Nutritional supplement
PNer plus™	Vitamin and other natural supplements	Neuropathic pain supplement
Pediasure®	Protein, vitamins, and other natural supplements	Nutritional supplement
Betafactor® capsules	Beta-glucan	Immune supplement
Brain speed Memory®	Blend of vitamins and minerals	Brain health
Pediasure®	Protein, vitamins, and other natural supplement	Nutritional supplement
Beneflora® probiotic	Lactobacillus acidophilus Bifidobacterium bifidum	Gastrointestinal health
Ferradol Food® Powder	Carbohydrates, proteins, iron calcium, zinc, vitamins	Nutrition supplement
Revital ®	Ginseng, vitamins, and minerals	Antioxidants and daily health supplements
Becadexamine®	Multivitamins	Nutritional supplement

4. Result

The differentiation of individuals' demands and attitudes towards food, reshaping of food supply, advanced technology in the food industry, and developments in nutrition science have paved the way for the discovery of new food and food products that can have positive effects on health. Today, nutrition science has turned to intensive research for the development of new food products that support the protection of health as well as daily nutrition. As a result, the concepts of nutraceutical and functional food emerged. Since nutraceuticals and functional foods make positive contributions to human and animal health, it is thought that with the consumption of these products, the society can be fed more healthily, the quality of life can be increased, and diseases can be prevented and treatment expenditures can be reduced. Therefore, the production and consumption of functional foods and nutraceuticals should be encouraged. More research on functional food and nutraceuticals should be conducted and supported to substantiate health claims on functional food and nutraceuticals, and international consensus based on objective and scientific criteria should be reached. In addition, seminars on the importance of functional food and nutraceuticals in healthy nutrition should be given to dietitians, doctors, nurses, and teachers working in the field of health and education, and the public should be made aware of this issue.

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FUNCTIONAL FOODS AND HEALTH CLAIMS

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1.   Introduction

The growing awareness of the close relationship between nutrition and human health has changed food preferences in modern countries, leading consumers to prefer one food over another to obtain a desired health outcome (1, 2). Functional foods are an ideal choice in this regard, as they aim to enhance quality of life by preventing dietary disorders (3). Even though there is no common explanation for "functional foods" and "nutraceuticals" because there is no controlled vocabulary or standard terminology, a "nutraceutical," since these two names are commonly used interchangeably, has been explained as *"A food or component of a food that has medical or health benefits, such as disease prevention and/or treatment"* (4). Doyon and Labrecque (2008) reviewd 26 different proposed explanations of "functional food" in the literature and came up with the proper definition following: *"A functional food is a traditional food or looks similar to one. It is part of a normal diet and is consumed regularly and in normal amounts. It has been shown to have health benefits, including reduce the risk of certain chronic diseases or have beneficial effects on certain target functions beyond fundamental nutritional functions."* (5). According to these explanations, both functional foods and nutraceuticals aim to improve mental and physiological activity, reduce disease risk, and treat health disorders to promote physiological functioning (health promotion) (6).

Functional food labeling statements or "claims" are crucial because they help customers understand the unique health advantages of these items and urge them to make the right dietary choices. Functional foods with health claims are perceived to be healthier to a lesser extent than those without any claims, as stated by the CLYMBOL project (the first cross-national study funded by

the European Commission to assess the role of health claims and symbols on consumer action and to compare the current state of food and beverage claims in Europe) (7). Furthermore, health claims are critical to the food industry since they are frequently used in food marketing and encouraging food firms to innovate and compete by ensuring that each product is properly labeled before being marketed (8).

In theory, all health claims in commercial food communications (labeling, presentation, advertising, and promotional campaigns) should be subject to complete and explicit regulation. However, there is no single regulatory structure for these claims. Differences in international food legislation make it difficult for the food sector to promote functional foods (9, 10). The authors' previous study intended to clarify the differences between the concepts and definitions of functional foods in the pioneering food-pharma sector, which includes functional foods, dietary supplements, and nutraceuticals (3).

## 2.   The Role of Health Claims in Health Promotion

Health claims are used to convey a health-promoting message to consumers, telling them which products to buy to achieve beneficial physiological effects on biological systems or lower the risk of disease (11). Individual products may provide clear, well-defined benefits, but balanced dietary advice, which focuses on the impact of the complete diet rather than individual products, is considerably different. The stated advantage of eating a balanced diet is a better chance of staying healthy and reducing the risk of lifestyle-related diseases, but the benefits are unclear and distant, and in most cases, no immediate benefits are seen or felt while working toward the objective (12). Functional foods, on the other hand, promise to deliver outcomes in a short amount of time. Some effects, such as reducing blood cholesterol levels, are easily quantifiable, while others, such as immunological improvement, depend heavily on belief in the stated impact. The issue is that there are no biomarkers that consumers can assess and utilize as indicators of impacts for many conceivable activities (13).

Physicians, consumer groups, and government officials have expressed concern about health claims. One question is whether customers view health claims as a solution to healthy eating without considering other factors (14). When Quaker Oats studied how people understand health claims, they found that individuals who saw an oatmeal or fiber claim on the packaging were not more inclined to believe they didn't need to pay attention to the rest of their diet

(15). However, a Finnish study that used a shopping list approach to indirectly quantify consumers' perceptions of functional food shoppers discovered that functional food shoppers were perceived as more innovative and disciplined than shoppers of comparable conventional products. Consumers with a fundamental list of products with a high health image were judged as more disciplined, in any case of their functional selections. Choosing functional foods took some discipline, but not nearly as much as buying traditionally healthy foods, implying that functional foods are seen as a less difficult option than healthy foods in general (16).

3. The International Regulatory Status of Health Claims

The Nutrition Labeling and Education Act governs the mandatory information that must be included on food and dietary supplement labels (e.g., manufacturer and distributor information) and nutrition labeling, which includes health claims (last amended 2016), codified in Title 21 of the Code of Federal Regulations (21 CFR) (1967) (last amended 2018). The European Food Safety Authority (EFSA) evaluates the validity of this evidence, and the European Commission makes decisions on the authorization of claims based on EFSA's recommendations. EFSA conducts a scientific evaluation of the evidence for the supporting the claim (17). This assessment includes three criteria outlined in previous evaluations of EFSA's assessment process: (1) the bioactive substance must be adequately described, (2) the suggested claim must involve a positive physiological benefit, and (3) it must be proved that the bioactive chemical has a causal relationship with the favorable physiological effect. (Figure 1).
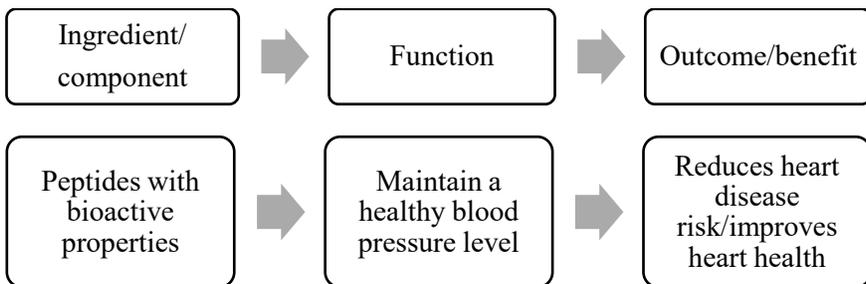
Fig. 1. Three different components can be used to create health claims, either alone or in various combinations. The result can be presented as promising a benefit (a positive outcome) or preventing a negative consequence (a negative outcome) (18).

3.1. *American Regulation*

Disease, the relational component, must be adequately characterized and focused on lowering disease risk, rather than curing, alleviating, treating, or preventing disease, as medicine does. A claim claiming a pain-relieving action to treat the signs of arthritis, for example, are not be considered a health claim since it implies that the food can be used as medicine. Only foods with the right nutritional profile can make health claims on their packaging. Alternatively, foods may include at least 10% of the daily value for one or more of the six essential nutrients, according to the Reference Amount Customarily Consumed (RACC) (protein, fiber, calcium, vitamin D, etc.). The meal in issue must meet this minimum nutritional content before any intentional addition of nutrients. Furthermore, health claims should be expressed in a way that (1) illustrates the proportional importance of the claimed health influence in respect to the overall daily diet and (2) is understandable to consumers (19).

"Authorized health claims" and "qualified health claims" are the two types of health claims. Approved health claims may imply or suggest foods help to prevent sickness or other health problems. To be accepted by FDA as an approved health claim, it should be based on a significant scientific agreement (SSA health claims) or an authoritative declaration from an appropriate U.S. government scientific body or the National Academy of Sciences or one of its branches (FDMA health claims). The definition of an SSA has been debated for a long time. The SSA standard applies to all approved health claims on food and dietary supplement labels (20).

Any food firm that wants to advertise its goods with recently created health claims that explains or implies recently created health effects must apply to FDA for premarket approval (21). Approved health claims are only allowed after a thorough evaluation of the scientific research. Randomized controlled clinical intervention trials provide the most trustworthy evidence (commonly referred to as the "gold standard") for proving the association between a specific food ingredient and health. Before a recently created health claim may be certified, a variable number of clinical intervention studies are necessary in each situation. For example, some clinical studies were sufficient to support approval of the health claim connected to soluble fiber from wheat and coronary heart disease ("Soluble fiber from foods such as [name of food] may reduce the risk of heart disease as part of a diet low in saturated fat and cholesterol"), whereas the health claim on wheat soluble fiber and coronary heart disease was approved. ("A diet

rich in soluble fiber from whole oats and low in saturated fat and cholesterol may lower the risk of heart disease.") (22). SSA health claims appear on both foods and supplements, whereas FDMA claims appear solely on foods (24). FDA has accepted 18 health claims, as stated in Table 1.

Table 1. List of authorized health claims based on Significant Scientific Agreement (SSA health claims) and those health claims based on authoritative statement from scientific bodies of the US Government, the National Academy of Sciences or any of its subdivisions (FDMA health claims) (34).

Component/ingredient-disease/health-related condition	Model claim statement(s)
SSA health claims	
Calcium and osteoporosis and calcium, vitamin D, and osteoporosis	"Adequate calcium throughout life, as part of a well-balanced diet, may reduce the risk of osteoporosis" and "Adequate calcium and vitamin D, as part of a well-balanced diet, along with physical activity, may reduce the risk of osteoporosis."
Dietary fat and cancer	"Development of cancer depends on many factors. A diet low in total fat may reduce the risk of some cancers".
Sodium and hypertension	"Diets low in sodium may reduce the risk of high blood pressure, a disease associated with many factors"
Dietary saturated fat and cholesterol and risk of coronary heart disease	"While many factors affect heart disease, diets low in saturated fat and cholesterol may reduce the risk of this disease".
Fiber-containing grain products, fruits, and vegetables and cancer	"Low fat diets rich in fiber-containing grain products, fruits, and vegetables may reduce the risk of some types of cancer, a disease associated with many factors".
Fruits, vegetables and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease	"Diets low in saturated fat and cholesterol and rich in fruits, vegetables, and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the

	risk of heart disease, a disease associated with many factors".
Fruits and vegetables and cancer	"Low fat diets rich in fruits and vegetables (food that are low in fat and may contain dietary fiber, vitamin A, or vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors".
Folate and neural tube defects	"Healthful diets with adequate folate may reduce a woman's risk of having a child with a brain or spinal cord defect".
Dietary non-cariogenic carbohydrate sweeteners and dental caries	"Frequent between-meal consumption of food high in sugars and starches promotes tooth decay. The sugar alcohols in [name of food] do not promote tooth decay" and "Does not promote tooth decay".
Soluble fiber from certain food and risk of coronary heart disease	"Soluble fiber from food such as [name of soluble fiber source and, if desired, name of food product], as part of a diet low in saturated fat and cholesterol, may reduce the risk heart disease. A serving of [name of food product] supplies _ grams of the [necessary daily dietary intake for the benefit] soluble fiber from [name of soluble fiber source] necessary per day to have this effect".
Soy protein and risk of coronary heart disease	"25 g of soy protein a day, as part of a low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies _ _ grams of soy protein", and "Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce

	the risk of heart disease. One serving of [name of food] provides _ _ grams of soy protein".
Plant sterol/stanol esters and risk of coronary heart disease	"Food containing at least 0.65 g per of vegetable oil sterol esters, eaten twice a day with meals for a daily total intake of least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies _ _ grams of vegetable oil sterol esters", and "Diets low in saturated fat and cholesterol that include two servings of food that provide a daily total of at least 3.4 g of plant stanol esters in two meals may reduce the risk of heart disease. A serving of [name of food] supplies _ _ grams of plant stanol esters".
FDMA health claims	
Whole Grain Food and Risk of Heart Disease and Certain Cancers	"Diets rich in whole grain food and other plant food and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers".
Whole Grain Food with Moderate Fat Content and Risk of Heart Disease	"Diets rich in whole grain food and other plant food, and low in total fat, saturated fat, and cholesterol may help reduce the risk of heart disease".
Potassium and the Risk of High Blood Pressure and Stroke	"Diets containing food that are a good source of potassium and that are low in sodium may reduce the risk of high blood pressure and stroke".
Fluoridated Water and Reduced Risk of Dental Caries	"Drinking fluoridated water may reduce the risk of [dental caries or tooth decay]".

Saturated Fat, Cholesterol, and Trans Fat, and Reduced Risk of Heart Disease	"Diets low in saturated fat and cholesterol, and as low as possible in trans fat, may reduce the risk of heart disease".
Substitution of Saturated Fat in the Diet with Unsaturated Fatty Acids and Reduced Risk of Heart Disease	"Replacing saturated fat with similar amounts of unsaturated fats may reduce the risk of heart disease. To achieve this benefit, total daily calories should not increase".

Eventually, qualified health claims are those made on food and dietary supplement labels that are backed up by scientific evidence. They must, however, be accompanied by a disclaimer because they do not meet the SSA's requirements (23). At this level, the FDA has established a classification system for health claims based on the relative weight of the scientific evidence supporting each claim. Scientific evidence is separated into three categories (B, C, and D) that show the level of evidence (moderate/good, low, and low, respectively) under this classification. Health claims must be labeled on functional foods with the proper wording depending on the category: "Although scientific evidence exists to support the claim, it is inconclusive..." (B or second level); (2) "Some scientific evidence suggests..." (C or third level); (3) "Very limited and preliminary scientific study shows... FDA believes that there is little scientific evidence to support this claim" (D or fourth level). The "A" category (first tier) includes all allowed health claims that match the SSA criteria, such as those permitted in Europe (24).

3.2. European Regulation

The licensing process for recently health claims is complicated since these claims must be backed up by enough scientific data, which necessitates a standardized assessment by EFSA. The main scientific basis for a health claim is published human studies that address and prove the link between the food or substance (to which the claim refers) and the health effect. As a result, a thorough examination of said human research is required, which must be completely clear and reflect all available scientific data. Unpublished human studies may also be allowed, but a detailed description of the methodology used to find them is required. Animal studies can serve as "supporting evidence" by establishing the claim's biological validity or elucidating the potential mechanism(s) through which the food or drug might have the claimed effect. Some research used to support a health

claim, in any case of technique or reporting, must be of high quality. Finally, a health claim will be approved for use on food labels if available scientific evidence shows that (1) the food or composite having the desired effect is fully defined and described, and (2) the quantity and pattern of consumption required to achieve that effect can be reasonably achieved through a balanced diet (25).

The European Union-funded REDICLAIM project was made to assist new requests in obtaining approval for recently created health claims. This European project contains papers such as guideline documents, assessments of the EFSA's scientific judgments on recently created health claims, and information from specialists participating in the application procedure. Food corporations must (1) justification for using health claims, (2) adhere to the specified conditions of use, and (3) ensure that foods bearing health claims have an accurate nutritional profile. Nutrition labeling is required on all items, even those with health claims (26).

If a claim is made for a chemical that isn't specified on the nutrition label, the amount of that chemical must be displayed in the same area of view as the nutrition label. These claims assist consumers in making informed food choices in order to eat healthy (27). Food manufacturers are typically interested in making health claims on their labels wherever possible, however there have been some documented problems and hurdles (28-30).

4.   The Commercial Functional Foods with Health Related-Claims

In industrialized cultures, the substantial market for functional foods reflects consumers' positive perceptions of these goods and the current trend toward healthier eating patterns. The estimated investment of more than $300 billion in the coming year reflects the promising future of the functional foods sector in developed countries, and it demonstrates the current trend toward innovation both in the food industry, and in the pharmaceutical sector, which has shown a strong interest in investing in functional foods in recent years. High healthcare spending due to patients with chronic conditions, faster development timelines, and cheaper product development costs are all key incentives for the pharmaceutical industry to promote functional food development (29, 31).

Consumer acceptance, which is assessed by a set of criteria classified into two groups: consumer-related features and product-related qualities, may be related to the market potential for functional foods with health claims (Figure. 2). The first group includes personal qualities like age, gender, health awareness,

familiarity, income, and education, as well as psychological aspects like health motivation, consumer attitude, and food phobia, as well as cultural and societal elements. When a sick family member and youngsters are present, as well as when doctors or nutritionists provide information, acceptance of functional foods increases. The another group includes price, flavor, brand, package characteristics, and labeling. The factors that have the most impact on customers' purchasing decisions include labeling, health incentive, and consumer attitudes toward functional foods. Consumers committing to improving and/or preserving their health are more interested in label information and are more likely to have a good behaviour toward purchasing functional foods, so these three components are partially interconnected (31-33).
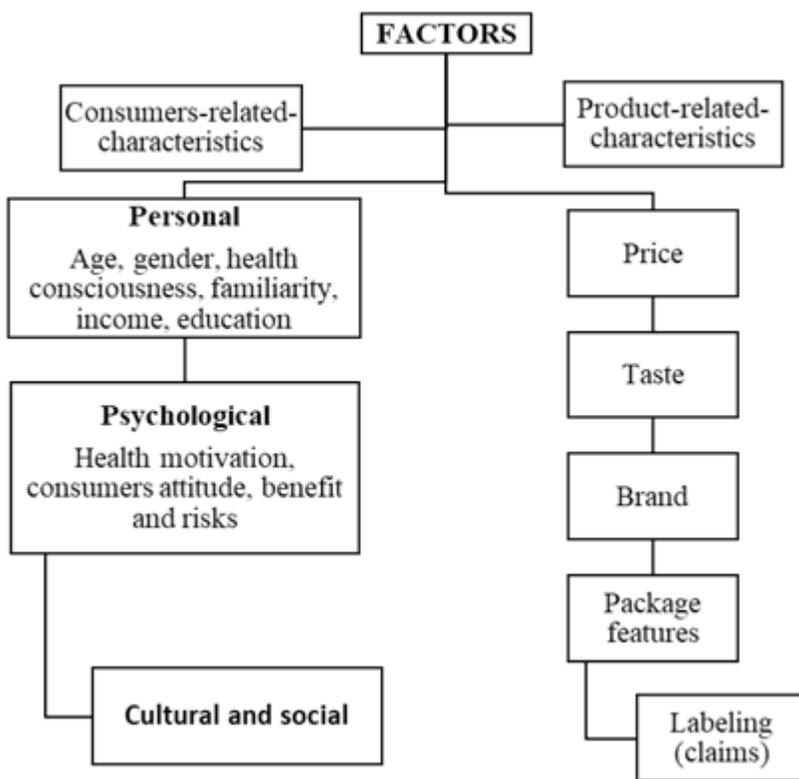
Fig. 2. General classification of factors influencing consumer preferences and acceptance of functional foods (34).

Consumers' food choices are positively influenced by health claims on food labels that have been approved by regulatory organizations. They also have the ability to build hedonic and sensory expectations that will influence future

interactions with the functional foods. Customers tend to look at the claim first and for longer than the other parts of the box, despite the fact that package design strategies (such as imagery) can capture consumer attention and make the functional foods stand out from the competitors. The CLYMBOL study found that packaging pictures did not convince buyers to choose a functional meal with a health claim more frequently (35).

On the one hand, consumer comprehension of the data provided by these claims is crucial. Consumers are more likely to buy functional foods if they understand and believe in the health benefits promised on the labels; nevertheless, this does not necessarily result in a final purchase. It's vital to note that health claims have a bigger influence on consumers buying a certain sort of product and are interested in nutrition. Consumers who do not have this objective are unlikely to buy a food product just because it has a health claim on the label (36).

Nineteen research revealed that nutrition knowledge has a substantial impact on consumer acceptability and buy aims of functional items with health claims. Alternatively, health motivation was discovered to be a factor. Consumers with a specified health purpose are more likely to choose a functional food with health claims. In general, it appears that customers who wish to make better their quality of life by eating healthier pay greater attention to product label health claims than other customers. The vast majority of customers with strong health motivations would be willing to pay a higher fee for functional foods with health claims, but not at the expense of flavor. The flavor of these items appears to have an important role in their adoption. When the taste of healthy functional foods is impaired, customers react negatively (37).

In conclusion, health claims can help customers make better-informed decisions by alerting them to the potential health impacts of using functional foods. Consumer views of these products, on the other hand, may be unpredictable. In order to effectively quantify and assess the impact of health claims on consumer behavior and attitudes toward functional foods with these claims, more study in this complex field is required (38).

It is difficult to predict the market potential for functional foods with health claims; nevertheless, a survey of customer sentiments toward these products could be a good place to start (36, 37). According to the twenty-three research collected and evaluated in Mogendi et al. (2016)'s systematic review, sociodemographic factors are just as important as those previously mentioned. Cultural and national distinctions are considerable (36). Americans consumers,

for example, are more inclined than Europeans to accept the concept of functional foods with health claims and are willing to include these goods in their daily diets. Because they are more interested in purchasing these products, customers from Cyprus, Poland, Netherlands, Sweden and Finland are more receptive than consumers from other European nations (Denmark, Italy, and Belgium) (38).

5. Conclusions

Health claims may be made on the labels of functional foods, which is an important means of informing consumers about the relationship between diet and health. Consumer purchasing decisions can be influenced by labeling, health appeal, and consumer attitudes toward functional foods. Customers are better protected by inconsistent claims since they are able to make more educated food choices. Consumer behavior toward functional foods is influenced by a number of factors and varies by nation, with acceptance of these items being easier in the United States than in Europe. To fully get the impact of health claims on consumer behavior and attitudes toward functional foods that make these claims, further research is needed.

The functional food industry has seen an increase in investment in recent years and appears to have a bright future. According to the authors' market study, most functional foods with health claims contain a lot of declarations on their labels, which matches the CLYMBOL initiative. The substantial number of these health claims include substances that are well-known to consumers (vitamins and minerals). This extensive study can be important in three ways: (1) assisting customers in making more educated buying choices about functional items, (2) assisting the food business in marketing its products internationally, and (3) assisting scientists in evaluating their everyday research.

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A BIOACTIVE COMPOUND: CAPSAICIN

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1.   Introduction

Capsaicin, which is used by humans and causes physiological changes in the organism, is a secondary metabolite. Secondary metabolites in plant-origin sources are bioactive compounds that has many functions in plant growth and development (1-3). These metabolites protect plants from bacteria, viruses, fungi, and insect pests as well as provide colour, taste, and odour qualities specific to each plant (2). Plant sources are foods rich in bioactive compounds. Bioactive components of plant origin, referred to as phytochemicals, are divided into three groups: Terpenes and terpenoids, alkaloids, and phenolic compounds (4-6). Bioactive compounds, which are generally esterified or bound to glycosides in plants, have an aromatic ring and at least one hydroxyl (-OH) group in their structure (7). Based on the structural differences of the aromatic rings, the number of -OH groups, and the bonds they have formed with various carbohydrates and organic acids, there are more

than 30000 different types of bioactive compounds and approximately 5000-10000 of them are included in our daily diet (1,8,9). Bioactive compounds in plants are found to reduce the incidence rates and risks of cardiovascular diseases and cancer, as well as age-related degenerative disorders such as brain and immunological dysfunction (10-13). Despite contradicting studies on the health benefits of bioactive compounds, it is stated that they have protective properties against oxidative stress caused by free radicals that effective in the aetiology of many diseases. Bioactive compounds exhibit a wide spectrum of antioxidant activities both inside and outside the living organism. There are multiple mechanisms of antioxidant activity, such as the reduction of the negative effect of reactive nitrogen species (nitrite and peroxynitrite) and reactive oxygen, retention of synthetic-free radicals and 1,1-diphenyl-2-picrylhydrazine (DPPH), the removal of lipid radicals, such as peroxyl radicals, forming in vivo and in foods as a result of lipid peroxidation, the formation of chelate with metals (Fe^{+3}, Cu^{+2}), as well as the activation of endogenous antioxidant enzymes, and assuming a protective role against deoxyribonucleic acid (DNA) damage caused by nitrites and peroxynitrites (1,6,12). Bioactive compounds react directly with free radicals and prevent them from reacting with other cell compounds (12,14-16). These compounds are also stated to serve as enzyme inhibitors and inducers, receptor inhibitors and inducers, and gene expression inhibitors (17).

Capsaicin is archaic and causes a burning sensation on the skin in case of contact. Capsaicin (CAP) (trans-8-methyl-N-vanillyl-6-none amide) is an alkaloid substance, and also an antioxidant that is the active ingredient of red-hot pepper. Capsaicin and other capsaicinoids have been extensively studied, particularly due to their use in different biomedical fields (18). The pepper (*Capsicum annuum* L.) is a plant that belongs to the Solanaceae family. It is also rich in vitamins A, B, C, and E, as well as carotene, polyphenols, flavonoids, minerals, and essential oils (19). Capsaicin, a bioactive molecule, is its main component (20). Capsaicin, in addition to regulating the digestive system, is used in the treatment of diseases, such as rheumatism, muscle pain, hypertension, and as protective folk medicine against cancer (21). The pepper plant, which is employed in the pharmaceutical industry, is also known to have bactericidal and insecticidal effects (22,23). Capsaicin has the molecular formula $C_{18}H_{27}NO_3$, its pungency is determined mostly by the benzene ring and modified by the acyl chain. Its molecular weight is 305.41 g. It is a crystalline, lipophilic, odourless, and colourless powder. Its melting point ranges from 57 to 66 °C. It is soluble

in alcohol, chloroform and benzene, slightly soluble in carbon disulfide, and insoluble in cold (10.3 mg/L at 25°C). Despite being held, frozen, and cooked, it preserves its original structure (Figure 1) (24,25).
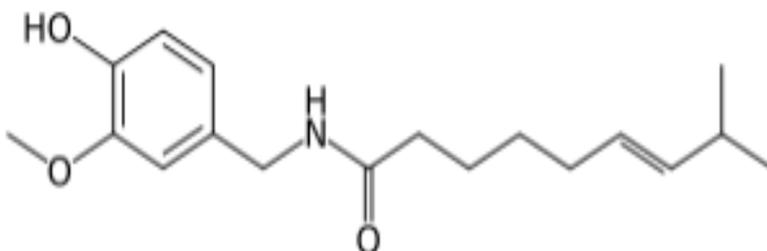
Figure 1. Chemical structure of capsaicin (24).

Red pepper production in Turkey is concentrated in the Mediterranean, Aegean, East Marmara, and South-eastern Anatolia regions, and according to the data of the Turkish Statistical Institute, 2.636.905 tons of red pepper were produced across an area of 119.869 decares between 2020 and 2021, and the per capita consumption was determined to be 24.7 kg (26). The use of red pepper as a common spice throughout the world has made it a basic ingredient in many cuisines and has given it different names depending on the regions where it is consumed. It is often called as "chile","-chilli" in Mexico and Central America, "aji" and "rocoto" in the Caribbean and Latin American countries, as well as it is called "pimiento" in Spanish, "red pepper and pepper" in the English language, and "pepper" in Italian, "piment" in French, and "paprika" in German and other northern European languages (27-29).

Capsaicin, which is the subject of this review, is the active ingredient of hot peppers and is an exogenous agonist of transient receptor, potential vanilloid 1 (TRPV1) receptor. TRPV1 receptors are expressed in cells of the nervous and immunological systems. It belongs to this group because its chemical properties are close to those of vanilloids (30). Due to the existence of a hydrophobic hydrocarbon tail, chemically purified capsaicin is a volatile, colorless, odourless, and crystalline compound that is insoluble in water but readily soluble in lipids and alcohol and is well absorbed up to 94% when administered topically or orally (31,32). Figure 2 illustrates the anatomical structure of red pepper.
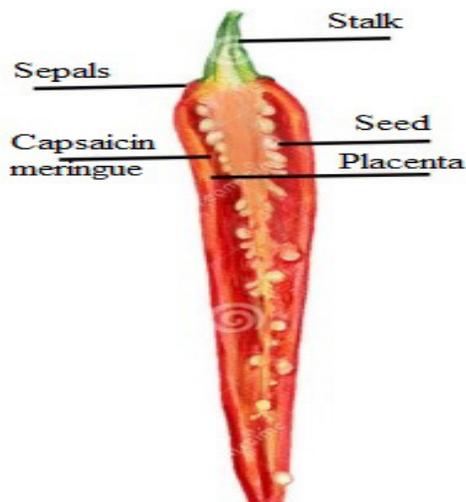
**Figure 2.** The anatomical structure of the pepper (33).

2. Metabolism of Capsaicin

Capsaicinoids are absorbed intragastrically at a rate of 85% and are metabolized in the liver before reaching the general circulation and extra-hepatic organs. In-vitro and in-vivo studies have indicated that capsaicinoids are metabolized by P450 enzymes through pathways, such as hydrolysis of the acid-amide bond and oxidative deamination of the formed vanillylamine, and hydroxylation of the vanillyl ring, possibly by epoxidation, as well as the formation of phenoxy radicals and capsaicinoid dimers by one-electron oxidation of the hydroxyl ring and oxidation of the terminal carbon of the sidechain. Capsaicin was observed to have rapidly been absorbed from the stomach and small intestines in the in-vivo and in-situ experiments on rats (34,35). Capsaicin metabolism in the human stratum corneum is also extremely rapid (36). In one study, 5 g of capsaicin and capsaicinoids were administered orally to healthy volunteers, resulting in a significant reduction in plasma glucose levels as well as an elevation in plasma insulin levels (37). Within one hour, a maximum distribution of 24.4% of administered capsaicin was observed in blood, liver, kidney, and intestine, which dropped significantly four days later until it was found (38).

3. Mechanism of Action of Capsaicin

Capsaicin binds mainly to the transient receptor potential vanilloid 1 (TRPV1) receptor found in sensory neurons (Figure 3) (28,39). TRPV1 is found primarily

on sensory nerve fibres, neurons, brain, heart, skeletal muscle, smooth muscle cells, liver, bladder, testis, kidney, intestine, epidermis keratinocytes, as well as glial cells, adipocytes, endothelial cells, pancreatic β cells, polymorphonuclear granulocytes, mast cells and macrophage tissues, and widely distributed throughout the body (40). The TRPV1 receptor is well-known for its major roles in inflammation, oxidative stress, and pain sensation. Temperature changes, physical erosion, pH variations, and endogenous lipids stimulate the receptor. TRPV1 is activated when capsaicin binds to it. The levels of sodium and intracellular calcium elevate, and a sensory neuronal depolarization cascade begins, allowing calcium ions to flow. When nociceptive sensory nerves depolarize along their fibres, the signals induce action potentials that propagate towards the spinal cord and brain. Capsaicin at high concentrations or repeated administration may produce a persistent local effect on cutaneous nociceptors. This condition, defined as capsaicin-induced dysfunction, reduces spontaneous activity and response to sensory stimuli (Figure 4). When capsaicin comes into contact with sensory neurons, it causes pain, inflammation, and a localized sensation of heat. Capsaicin also inhibits electron chain transport in high concentrations, making mitochondria dysfunctional and reducing direct pain transmission by desensitizing sensory afferent axons. Capsaicin stimulates the analgesic response by desensitizing sensory neurons when applied locally to the skin. The sensation of heat, burning, stinging, or itching is caused by topical exposure (28,39,41,42).
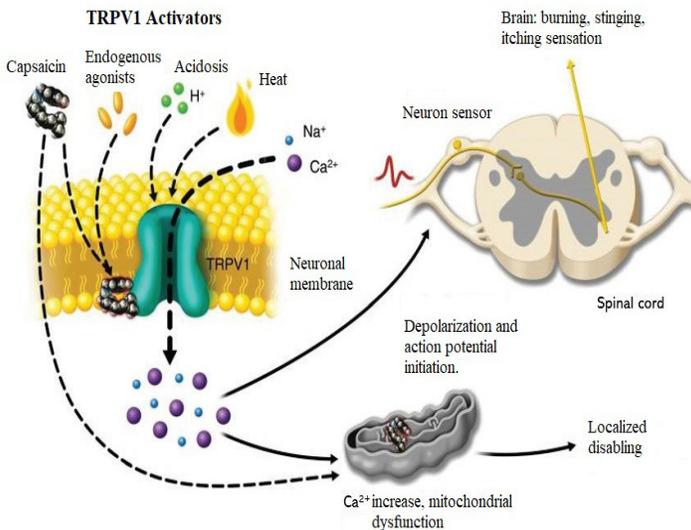
Figure 3. Activation of TRPV1 by capsaicin (39,41).
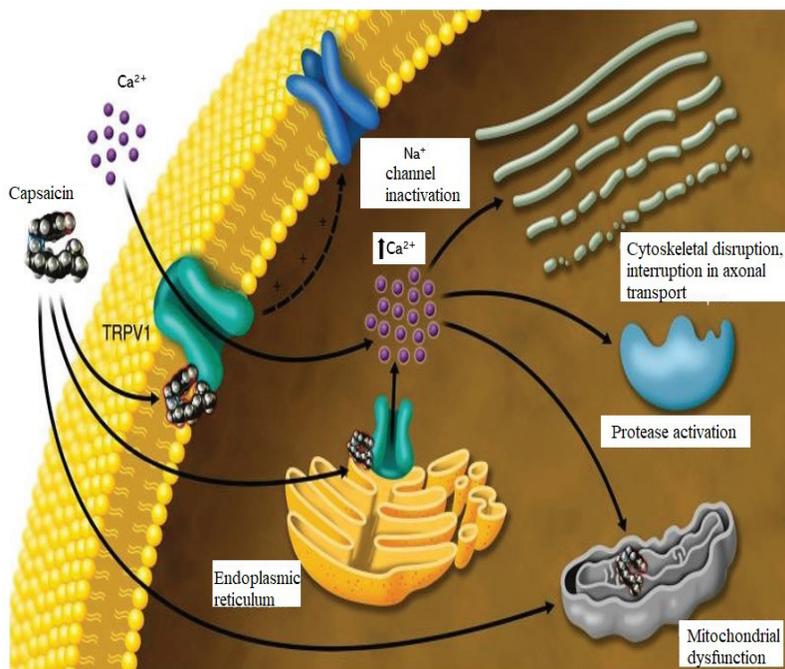
Figure 4. Capsaicin-induced dysfunctional mechanisms (39,41).

4.   Effects of Capsaicin

The physiological effects of capsaicin, which is pharmacologically important, have been examined in many studies. On the gastrointestinal tract, it has thermogenic, cardioprotective, antilithogenic, anti-inflammatory, and pain blocker effects. The effects of capsaicin are also related to the activity of calcitonin gene-related peptides, serotonin, somatostatin and P substances. Application of capsaicin to the skin has been proven to reduce pain in arthritis, post-surgical neuralgia, diabetic neuropathy, and psoriasis. Capsaicin stimulates gastric mucosal blood flow specifically, which contributes to the prevention and healing of stomach ulcers. Several in vitro and in vivo studies have determined the antioxidant and anti-inflammatory properties of capsaicin. Cholesterol prevents gallstone formation. It also ensures the preservation of the integrity of erythrocytes in hypercholesterolemia (29,43). It has also been reported that its anti-inflammatory and analgesic effects are related to the inhibition of cyclooxygenases (COX) (44,45).

One of the most important properties of capsaicin is its antioxidant activity. Oxidative stress, which develops particularly in the development of

cancer, cardiovascular disorders, atherosclerosis, and neurological disorders such as Parkinson's and Alzheimer's, has emerged as a major health concern (46). The studies have argued that capsaicin consumption rises in certain parts of the world due to its antioxidant properties (47), and it is effective in both the treatment and prevention of neurodegenerative disorders (48). Capsaicin has been proven to inhibit lipid oxidation by lowering oxidative stress caused by scavenging free radicals (49-51). The level of antioxidants in the blood and brain was observed to have raised (52). Capsaicin derivatives have been observed to protect linoleic acid against free radical damage by inhibiting autoxidation as well as iron or EDTA-induced oxidation. Capsaicin prevents the formation of reactive oxygen species and the activation of nuclear factor-kappa B (NF-KB) and activator protein-1 (AP-1) induced by phorbol ester, as well as inhibits the induction of apoptosis and electron transfer into the mitochondrial complex (53,54). Furthermore, various studies have indicated that it inhibits lipid peroxidation and scavenges -OH radicals in water, ethanol, and 1-1'-diphenyl-2-picrylhydrazyl (DPPH) radicals in membranes (51). A study reported that the inclusion of Cemele pepper in the daily diet in both fresh and dried form may be important for satisfying some of the antioxidant needs of the organism (55). The study by Nur et al., revealed that capsaicin was effective on the immunohistochemistry and gene expression distribution of transforming growth factor-beta 2 (TGFβ2) in the testis, suggesting that it is also functional on cellular functions and embryonic development (56). Capsaicin has been identified to reduce the level of antioxidants, such as glutathione peroxidase 1 (GPx 1) in experimentally induced diabetes (57).

Capsaicin blocks local pain stimuli from reaching the brain by depleting the substance P, which is found in neurons and contributes to the transmission of pain stimuli from the periphery to the central nervous system, and diminishing the sensitivity of the skin and joint tissue to pain. Capsaicin has been proven to be effective in the transmission of pain stimuli in the central nervous system, as well as in the reduction of substance P, which is known to have a role in inflammation. The substance P is a neurotransmitter that is released by sensory C-fibres. Capsaicin is a specific antagonist of substance P. Thus, capsaicin extracts are used topically to alleviate pain in nerve damage and shingles that cause extreme pain (58).

The results of studies to determine the carcinogenic effect of capsaicin may differ from each other. Standardized protocols have begun to demonstrate that the carcinogenic quality of capsaicin is quite weak, and its purity level is

important as of 2000 even though this molecule is referred to as a "sharp double-edged knife" (59). Capsaicin has been determined to inhibit the proliferation of leukaemia cells, lead to apoptosis in lung cancer cells and human hepatoma HepG2 cells, and raise the apoptosis rate in some prostate cancer cells (60). Capsaicin is included in a significant therapeutic agent class due to its anti-proliferative impact on xenograft mouse models and cancer cell lines by blocking the cell cycle in G0/G1 phases and inducing tumour cell apoptosis (61). The proposed mechanism for the effect of capsaicin on cancer cells is that the cell that has been exposed to capsaicin suspends its progression throughout the cell cycle, which accordingly triggers the apoptotic processes to take place (62).

Capsaicin has beneficial effects on the cardiovascular system (63,64). It is widely known that the cardiovascular system has an abundance of capsaicin-sensitive sensory nerves, which play a significant role in the regulation of cardiovascular function through the release of multiple neurotransmitters, such as CGRP, Substance P, and others. Laboratory studies have revealed that CGRP has a protective effect on cardiovascular functions (65-67). Capsaicin can stimulate the release of CGRP by activating TRPV1, and thus, it has the potential to have beneficial effects on cardiovascular functions (64).

Capsaicin included in human diets reduces the blood-glucose level and raises the amount of insulin produced after oral administration of the compound during a glucose tolerance test. It was found that capsaicin was correlated with maintaining the level of insulin in the blood, and thus, lowering the blood glucose level (38). Gestational diabetes mellitus (GDM) refers to a condition when a hormone that is produced by the placenta inhibits the body's ability to make effective use of insulin. This prevents glucose from being absorbed by cells, which leads to an increase in its level in the blood. GDM can raise health risks for both the women and their babies in the future. Many women who have GDM suffer from pregnancy-related complications, such as high blood pressure and babies with high body weight. The effect of capsaicin supplementation on blood glucose, lipid metabolism, and pregnancy outcomes in women with GDM was investigated and positive findings were obtained (68). In women with GDM, a chilli supplementation that contained capsaicin regularly treated postprandial hyperglycaemia and hyperinsulinemia, as well as fasting lipid metabolism disorders, and reduced the incidence of the delivery of babies with high live weight (68). Capsaicin can lower glucose intolerance in obesity by inhibiting the inflammatory responses of adipose tissue. Capsaicin intake in human diets can reduce obesity caused by induced glucose intolerance and raise fatty acid

oxidation in adipose and liver tissues, which are significant peripheral tissues affecting insulin resistance (69).

Studies on its anti-neuroinflammatory effects have reported that capsaicin can inhibit the expression of the pro-inflammatory transcription factor, which regulates genes related to cell survival/proliferation and inflammation, and nuclear factor kappa B (NF-κB). A study indicated that capsaicin inhibited NF-κB and TNF activation in prostate cancer cells (70). Furthermore, another study demonstrated that capsaicin inhibited tumour cell growth by reducing superoxide radical anion levels, whereas capsaicin modulated the activation of NF-KB that were induced by the reactive oxygen species, consequently reducing cancer cell proliferation (23,71). Capsaicin, also, has been reported to have an anti-inflammatory effect in stimulated macrophages by reducing the expression of NF-kB in immune cells (72). In an in vitro cell culture study, incubating Schwann cells with capsaicin significantly reduced MHC-II production that was triggered by interferon-gamma as well as the expression of toll-like receptor-4 and intercellular adhesion molecule-1 mRNA (21). Also, it has been demonstrated that capsaicin reduced the expression of nitric oxide, TNF-α, and IL-1β depending on dose by suppressing NF-κB activation from BV-2 microglial cells, which were stimulated by lipopolysaccharide (65).
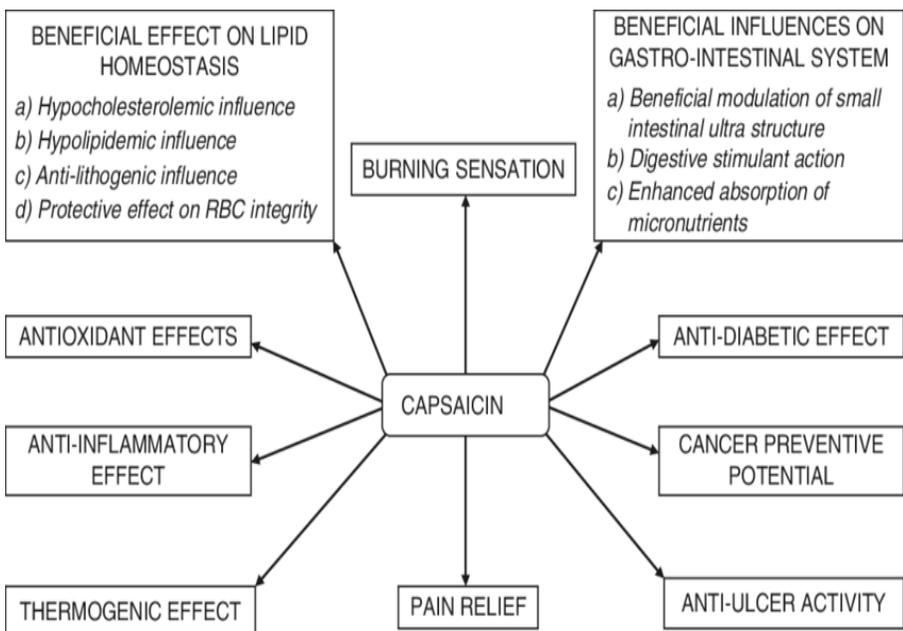
Figure 5. Summary of the diverse physiological effects of capsaicin (73).

5.   Conclusion

It has been determined that nutrition with fruits and vegetables abundant in bioactive compounds prevents chronic diseases in particular (74). The circulatory system (cardiovascular) related deaths take the first place. Diet rich in bioactive components reduces this risk. The mortality rate is 4-5% in those fed with a Japanese and Mediterranean diet rich in bioactive components. However, in Northern Europe, which is mostly fed with fast food, the death rate is around 15% (75). Aside from industrial applications, capsaicin compounds are widely utilized in the production of tomato paste and sauce materials as food, as well as in medicine for their anticarcinogenic, antioxidant, antihyperlipidemic, and analgesic properties, and in cosmetics for their anti-hair loss effects (76-78). It can easily permeate lipid bilayers and diffuse passively across cellular membranes due to its relatively high lipophilicity (23). An increase in daily intake of bioactive compounds obtained from plant sources will be an effective method in the avoidance of possible discomfort and diseases. Recently, increasing data reported that capsaicin has therapeutic effects in problems such as multifactorial metabolic disorders, cardiovascular diseases, obesity control, diabetes mellitus, cancers, dermatological conditions and neurogenic bladder, as well as has analgesic, antipruritic, anti-inflammatory, anti-apoptotic, antioxidant and neuroprotective effects (22). Further studies research is required to clarify the mechanism of action and biological activities of the capsaicin molecule and to determine the efficacy, toxicity, and safety of long-term use. Due to its high lipophilic structure and ease of passing through membranes, it would be possible to provide minimal side effects while bringing maximum benefit by establishing the dosages to be utilized in different diseases or different body parts while considering the dose-benefit correlation. Furthermore, while capsaicin is not directly beneficial in biomedical and medical applications, it can be designed as a carrier structure to assure the transport of various molecules into the cell by taking advantage of its easy passage and absorption feature through the membranes, and it would also be able to reduce the adverse effects of medications used to treat a variety of disorders.

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USE OF HERBS AND SPICES AS NATURAL ANTIOXIDANTS IN FOODS

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1. Introduction

Known as one of the causes of food spoilage, oxidation happens as a result of oxygen in the air impacting food components such as fats, carbohydrates, and proteins (1). Besides, environmental factors such as light, temperature, metal ions such as iron and copper, some pigments and the degree of unsaturation of fats are also the reasons that affect and accelerate oxidation (1, 2, 3, 4). Oxidation results in some undesirable sensory changes and quality losses in foods. Oxidation is accompanied by changes in the smell, taste and aroma of food, especially bitterness and rancid taste in fatty foods. This also triggers discoloration of the product and leads to changes in the structure and texture of the food. Along with the significant losses in the nutritional values of foods that have been exposed to oxidation, metabolites are also formed in their structures which are extremely harmful to human health.

Depending on the ratio of components in food, oxidation happens in fats, proteins and carbohydrates. Causing a bad taste, bitterness and aroma loss in food, lipid oxidation is a chemical chain reaction happening as a result of the degradation of the fatty acids present in the food component. This chemical reaction continues by self-catalyzing by binding oxygen to the octet gaps in polyunsaturated fats with the impact of various catalysts (5). Conditions that

are effective in the formation of lipid oxidation in foods include water activity, pH, temperature, light, metals contained in the food or contaminated during the subsequent application, enzymes, light and degradation, fatty acid composition of food, degree of unsaturation of fatty acids and amount of phospholipids, presence of antioxidant substances, processes applied during food processing and storage conditions (6). Even though the type of oxidation in foods is shaped depending on the component of the food, there is a close link between them that affect each other. Hydroperoxides and malonaldehydes formed after lipid oxidation bind to protein residues, which also leads to the oxidation of proteins (7, 8). Studies reveal that the free radicals formed as a result of the breakdown of polyunsaturated fatty acids as a result of lipid oxidation cause changes in the structure of the protein (9). Along with protein oxidation, protein polymers and amino acid modifications are observed, and these changes happen in a certain sequence in the form of chain reactions, as in lipid oxidation (10, 11). Oxidation of myofibrillar proteins results in a decrease in the number of essential amino acids and the digestibility of proteins as well as losses in the nutritional value of the product, undesirable color changes and loss of structural quality (12, 13). The oxidation of carbohydrates in foods with a high carbohydrate content leads to the formation of an undesirable color and aroma in foods. Maillard and enzyme reactions, high heat and oxidation of natural pigments play an active role in carbohydrate oxidation developing in foods.

If the factors that lead to or accelerate oxidation are eliminated, oxidation will also disappear. But in practice, it is very difficult to prevent oxidation without adding an external substance. Antioxidants and synergists are used as additives in cases where physical and technological methods cannot prevent oxidation (3, 14). Antioxidants are added to foods for reasons such as extending the shelf life of foods and preventing rancidity, as well as neutralizing free radicals formed as a result of oxidation.

2.   Antioxidants

Antioxidants are substances used during the production, storage, transportation and marketing of foodstuffs to prevent spoilage and bitterness of food. Antioxidants do not make any changes to the quality, taste and smell of food and do not eliminate rancid products or reverse oxidation. Antioxidants are added to foods to delay or prevent the formation of oxidation. The desired quality in food products can only be achieved by providing good raw materials, correct production methods, appropriate packaging and storage conditions. For the

proper and effective use of antioxidants, it is necessary to know the chemistry of vegetable and animal fats, the oxidation mechanism and the functions of the antioxidant used very well, and to add the antioxidant to the food before oxidation begins (15, 16). A good antioxidant should have the following features (17).

- They should be physiologically harmless
- They should not affect the smell, taste and appearance of fat and fatty products.
- They should not affect the oil and the foods prepared with it during cooking and should remain active.
- They should be sufficiently soluble in oil and be able to mix with it.
- They should be effective in small concentrations
- They should be easy to obtain and inexpensive.

Processes such as heat, shredding, refining, adding, packaging and storage applied during the processing of foods significantly affect the antioxidant (AO) capacity of foods. Heat treatments cause the oxidation of compounds with antioxidant capacity such as ascorbic acid. After understanding the protective effects of antioxidant compounds in foods and their importance for health, maximum attention was paid to the protection of natural antioxidants in foods during processing. In addition, their use in foods for protective purposes has gained importance and has become widespread recently. Antioxidants are frequently used in the food industry to prevent the oxidation of vegetable oils. A study has determined that the peroxide value of vegetable oils with antioxidants (caffeic acid, etc.) after frying was quite low compared to oils without antioxidants (18). It was also determined that using antioxidants in milk reduced the loss of vitamins during storage.

Antioxidants are divided into synthetic and natural antioxidants. Synthetic antioxidants are generally used to extend the storage period of industrial foods. However, many researchers point out that some synthetic antioxidants such as butyl hydroxytoluene (BHT), butyl hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) and propyl gallates (PG), which have been used in food processing for a long time, have carcinogenic and teratogenic effects in living organisms (19, 20, 21). For this reason, the interest in natural antioxidants and the transition from synthetic to natural products has become quite widespread recently in terms of healthy life.

3. Natural antioxidants used in foods

3.1 *Ascorbic acid and derivatives*

Found naturally in many food ingredients, ascorbic acid prevents the reaction of amines in the environment with nitrite and nitrate. Ascorbic acid and sodium ascorbate are particularly used to prevent the conversion of nitrite and nitrate, which are added in meat products as antimicrobial agents, to nitrosamine, a carcinogenic compound, by combining with free amine compounds in meat (22).

3.2 *Nordihydroguaiaretic acid (NDGA)*

NDGA is a natural antioxidant derived from the plant *Larrea divaricata* (Bushy shrub). It can also be produced synthetically. This product is especially used in essential oils, bakery products, fish and lard (23, 24).

3.3 *Tocopherols*

Found in plants, tocopherols are the most widely known and widely used natural antioxidants (25). The bioavailability of ascorbic acid decreases from 100% at low doses (<100mg) to up to 15% at high doses (>10g). The bioavailability of tocopherols with vitamin E effect can be up to 95%, while that of carotenoids can be down to less than 15%, although it is not known exactly (19, 21).

3.4 *Amino Acids, Peptides, Proteins*

Some studies indicate that amino acids such as Tryptophan and some peptides can be used as antioxidants in salami, sausage and dairy products (24).

3.5 *Natural enzymes found in foods*

Enzymes such as catalase, peroxide dismutase, glutathione peroxidase, ascorbate peroxidase, superoxide dismutase, glutathione reductase, which are found in the natural structure of the food, may exert a natural antioxidant effect on the food. Enzymatic AO systems biochemically neutralize superoxide radicals and reactive compounds (19, 21).

3.6 *Vitamines*

Certain vitamins with antioxidant properties also act by reducing free radicals (21). For example, vitamin E traps and neutralizes peroxyl radicals formed during lipid oxidation and thus prevents autooxidation. However, it then turns

into a radical, and this radical is reduced by vitamin C and converted back into vitamin E. The resulting vitamin C radical is also inactivated by body mechanisms, in particular by ascorbate peroxidase (19, 26, 27).

3.7 Carotenoids

It has been revealed that carotenoid compounds such as deastakisin, tunaxanthin, β-carotene, and 4-hydroxyechinenone, which are effective in the color formation of vegetables and fruits, are also found in many animal tissues (28,29). These carotenoid compounds are found especially in crustaceans. These compounds, which form the carotenoid protein structure by combining with proteins in invertebrates, also form the structure of the shell. For example, red crabs contain carotenoids such as β-carotene and astaxanthin. The 1-2-diamino 1-1-(o-hydroxy phenyl) propene compound, which is known for its antioxidant properties, is abundant in the shells of shrimps (30).

3.8 Hormones

It has been found that especially foods containing melatonin have high antioxidant activity and prevent oxidative damage caused by lipid peroxidation. In addition, it was revealed that melatonin prevents oxidative damage caused by lipid peroxidation in tissues by stimulating the activity of many enzymes. Studies also have reported that consuming foods containing melatonin such as almonds, hazelnuts, walnuts, chamomile tea and sour cherry will be effective in increasing antioxidant capacity (31, 32, 33).

3.9 Minerals

Minerals such as selenium, copper, zinc and manganese taken with food enter the structure of antioxidant enzymes (enzymatic antioxidants) in the body and act as catalysts and activators of these enzymes and provide indirect antioxidant effects (34, 35).

4. Herbs and Spices

The use of plants and herbal extracts as preservatives in foods has gained importance along with the increasing concern of people about the use of synthetic additives. Spices used in foods not only improve the sensory quality but also extend their shelf life by providing antimicrobial and antioxidant effects. Many herbs and spices are used experimentally to prevent bacterial and

oxidative deterioration in foods. Most of the herbs and spices with antioxidant properties belong to the Labiatae family. There are over 3000 species belonging to the Labiatae (Lamiaceae) family. Plants belonging to the Labiatae family, which grow naturally in Mediterranean countries, are widely used in medicine, perfumery, cosmetics and food industries (36). These plants have important physiological activities (antioxidant and antimicrobial) especially because they contain terpenic compounds (mono-, di-, triterpenes), flavonoids and phenolic acids. Flavonoids and phenolic compounds found in leaves, flowers and woody parts of such plants and spices prevent lipids, proteins and carbohydrates from being oxidized by free radicals (37). These phenolic compounds scavenge free radicals and show antioxidant activity by forming compounds with metal ions and thus prevent the formation of singlet oxygen (38, 39, 40, 41). Since the chemical components of herbs and spices are different, their antioxidant effects are also different from each other (42, 43). The main components of some herbs and spices are shown in Table 1. (44, 45, 46, 47, 48, 49, 50, 51).

Table 1. Active Substances Isolated from Some Herbs and Spices

Herbs and spices	Systematic name	Active ingredient
Rosemary	*Rosemarinus officinalis*	Carnosic acid carnosol, rosmarinic acid rosmanol, Borneol, α-Pinene, β-Pinene, 1,8-Cineole, δ-Terpinene
Sage	*Salvia officinalis*	Thujon, cineol, linalool, borneol, salven, pinen, camphor, estrogen-like substances
Thyme	*Thymus vulgaris*	Tyhmol, karvakrol, p-cunen
Clove	*Eugenia caryophyllata*	Eugenol, gallates
Turmeric	*Curcuma longa L.*	Curcumin
Black pepper	*Piper nigrum*	Phenolic amides, flavonides
Black cumin	*Nigella sativa*	p-Simen, karvakrol, t-Anethol, 4-terpinol, longifolin, timokinon, dihidrotimokinon, timol
Bay	*Laurus nobilis*	1,8-cineole, a-terpinyl acetate, sabinene
Sodgrass	*Melissa officinalis*	Neral (citral), citronellal, citronellol, nerol acetate, isogeraniol, geranyl acetate
Mint	*Mentha sp.*	Menthol, menthone, carvone, hesperidin
Dill	*Anethum graveolens L.*	Karvon, limonen
Green tea	*Camellia sinensis*	Epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG), quercetin, kaemferol, myrcetin
Hawthorn	*Crataegus sp.*	Quercetin, vitexin, apigenin, rutin, oligomeric proanthocyanidins, triterpene, organic acids, vitamin C, sterols

5. Herbs and Spices Used as Antioxidants in Food

Flavonoids and other phenolic compounds that serve as antioxidants in plants are mostly found in the leaves, flowers and woody parts of the plant (52). Therefore, leaves and flower parts of aromatic plants can be dried and used as drugs (53) or they can be used as essential oil extracts obtained by methods such as extraction and distillation (54). Antioxidant activities of plants, spices and their extracts on foods can be achieved by different application methods. These methods are;

- Adding the aforementioned plants or their extracts as an additive to poultry, ruminant or fish feed rations,
- Adding these herbs, spices or extracts during the processing and production of food,
- Ensuring the integration of these herbal additives and foodstuffs with different application methods during the packaging

Recently, many plants rich in phenolic compounds have been used as preservatives in foods. Rosemary (*Rosmarinus officinalis*), one of the most effective antioxidant agents used in foods, is the only commercial product available as an antioxidant in Europe and the USA (55). It has been reported in many studies that the extract obtained from rosemary leaves can be used as an antioxidant agent in foodstuff (56, 57). These studies also revealed that the compound showing the strongest antioxidant activity of Rosemary extract was carnosic acid, and this compound showed 7 times higher activity than the synthetic antioxidants BHT and BHA (57). In their study, Akgül and Ayar (42) stated that the plant with the highest antioxidant effect in sunflower oil among the other 31 plants they tried was rosemary. In another study, it was determined that the most effective antioxidant agent in meatballs stored for 12 days by adding rosemary, orange and lemon extracts was rosemary (58). In another study, it was reported that rosemary extract was as effective as synthetic antioxidants (BHA, BHT) in cooked pork, and showed higher antioxidant activity in raw frozen pork (59). McCarthy et al. (60) investigated the antioxidant effects of raw and cooked pork patties by adding aloe vera, fenugreek, ginseng, mustard, rosemary, sage, soy protein, tea catechin, and whey protein concentrate. They reported that catechin and rosemary were more effective on oxidative stability compared to other spices and additives. The impact of rosemary extract on minced meat and filets of hake and salmon was investigated and Malondialdehyde (MDA) levels in both minced meat and filets of both fish species were found to be significantly reduced compared to the control group (61). It has been reported that rosemary extract applied in different doses to cooked turkey meats increases lipid stability during storage in the refrigerator for 13 days (62). In another study, it was reported that ethanol extracts of white peony, red peony, sappanwood tree, Moutan peony, rosemary and angelica plants added to raw or cooked meatballs at a rate of 0.25% were effective on oxidative stability during storage (63). In a study examining the antioxidant activity at different temperatures by adding rosemary extract to chicken sausages, it was determined that rosemary extract

showed a high antioxidative effect at all temperatures (64). In another study, it was reported that the lipid oxidation and color change of ground and shredded beef were slowed by the addition of rosemary (65). Lopez-Bote et al. (66) determined that rosemary extract and α-tocopherol were equally effective in preventing lipid oxidation in chicken meat (breast and thigh) stored at -20 0C for 6 days. However, they found that α-tocopherol showed a stronger antioxidant effect than rosemary extract when chicken meat was stored for a long time, such as 4 months. In another study, Galobart et al. (67) determined that α-tocopherol inhibited lipid peroxidation in eggs enriched with omega-3 fatty acids, but the same effect was not in question for rosemary extract. Similarly, Harmankaya and Vatansever (68) stated that the antioxidant effects of rosemary and clove oils during the preservation of chicken thighs were insufficient. This may be due to the fact that the genotypic characteristics of the essential oils used, the geographical regions where they are grown, the climatic characteristics of these regions and the collection dates are different, even if they are obtained from the same type of plants. In addition, the method of obtaining the essential oil, the type of solvent used, which part of the plant is used to obtain the essential oil, whether this part is pounded or not, and whether the plant is dry or wet can cause changes in the oil composition (69). Apart from these, water, light and vegetation period are the factors affecting the type and amount of antioxidant compounds of the plant (70). In addition, the amount of dose applied to the food is one of the factors affecting the antioxidant activity. As a matter of fact, the intense aroma and smell of rosemary is not a problem that affects the amount of use; however, the USA and Japan have produced commercial preparations of rosemary that are colorless, tasteless, odorless and also have strong antioxidant effects (71).

Another plant with strong antioxidant properties is cloves. Eugenol, which is found in the composition of cloves, constitutes a large part of the clove extract and is the antioxidative element of the plant in question. Cloves show a strong effect as synthetic antioxidants such as BHT and BHA, which are frequently used in foods (72). Studies have reported that the antioxidant activity of cloves is higher than that of many plants. In one of these studies, it was determined that the antioxidant activity of clove essential oil on cottonseed oil was higher than that of thyme essential oil (73). Similarly, in another study investigating the antioxidant effect of plants on meat fat, it has been reported that clove is more effective than sage, thyme and ginger, and this situation varies with the applied dose (74). Contrary to these studies, in a study in which clove oil at

different concentrations was applied to chicken legs during the packaging phase, it was determined that there was no oxidative decline in chicken legs during the storage period.

In studies investigating lipid oxidation in frozen chicken and turkey meat, it has been reported that thyme oil reduces oxidation (54,75). It was stated that MDA levels in breast and thigh meats of broiler chickens fed with thyme essential oil or α-tocopherol acetate decreased as the amount of thyme essential oil increased. However, in the same study, it was reported that the antioxidant effect of thyme essential oil was lower than that of vitamin E (76). Bostoglou et al. (77) reported that lipid oxidation in liquid egg yolk could be controlled by adding thyme to the mixed feed. In a study, it was reported that the addition of thyme extract had a high antioxidant effect on meatballs kept in cold storage for days (78).

In another study, it was reported that the addition of thyme in fresh cheddar cheese produced with the addition of some herbs and spices had an antioxidant effect on cheddar cheeses (79). In a study conducted to determine the antioxidant properties of the Satureja (thyme) plant species in butter, it was revealed that this species has a strong antioxidant effect in butter because it contains thymol, carvacrol, and p-cemen (80).

Thymoquinone, which is found in the composition of black cumin, which is used to flavor foods, is a compound with a very important antioxidant effect. (81, 82) In a study, it has been reported that the antioxidant property of black cumin is not only dependent on the thymoquinone in its component, but also other components such as carvacrol play an important role in terms of antioxidant activity (83, 84). In a study investigating the antioxidant properties of essential oils obtained from black cumin seeds grown in Tunisia, it has been determined that components such as p-cymene, γ-terpine, thymoquinone, β-pinene, carvacrol, terpinen-4-ol and longifolene have antioxidative effects and that these components can be used as preservatives in foods (85). In a study examining the antioxidant capacity of black cumin seeds (*Nigella sativa L.)* using different methods, it was found that black cumin seeds showed better activity compared to synthetic antioxidants (86). Sultan et al. (87) determined in their study that black cumin essential oil has a higher antioxidant effect than fixed oil.

In a study, it was reported that lipid oxidation was significantly reduced in breast and thigh meats stored for a long time (4 months) by adding 500 mg/kg of sage extract to broiler feed (66). Kaya and Turgut (88) reported that the

addition of different levels of sage, thyme, peppermint extract and vitamin E to laying hen rations significantly slowed the formation of TBARS in eggs. In another study examining the effects of spices on oxidation by adding spices to meatballs alone or in a mixture, it was reported that the use of sage alone was more effective than a spice mixture (89).

Oleuropein and other phenolic compounds in olive leaf extract can be used as natural antioxidants in foods due to their high antioxidant activity (90). It has been reported that the antioxidant capacity of oils can be increased by adding olive leaf extract and hydrolysates to refined cooking oils instead of synthetic antioxidants (91). In one study, it was stated that olive leaf extract applied to corn oil increased the antioxidant capacity 14 times (92). It has also been reported that olive leaf extract and oleuropein can be used as a natural preservative in meat products (93) and in a related study, it was reported that oxidative stability increased during the storage process in pork minced meat coated with an active film containing olive leaf extract (94). Another study reported that the addition of olive leaf extract to yogurts with apricots increased antioxidant capacity (95).

In a study, it was determined that marjoram essential oil added to the ration of lambs showed a strong antioxidative effect by reducing lipid oxidation in meat (96). In another study, Florou-Paneri et al. (97) added marjoram oil to the diet of laying hens and they determined that the lipid oxidation in the egg yolk of the added group was lower than that of the control group.

Sumac (*Rhus coriaria*) also shows a high antioxidant effect thanks to the active fractions, anthocyanins and tannins in its structure (98). In a study investigating the antioxidant capacities of some spices used in food production, it was determined that the sumac plant has a high antioxidant capacity. In the same study, it was reported that due to the high phenolic compounds it contains, sumac spice can be used as an antioxidant in functional foods and many diseases such as cancer and heart diseases can be prevented with its consumption with food.

It has been reported in studies that the use of spices and extracts in dairy products increases antioxidant capacity (99). In a similar study, probiotic yogurts were produced with the addition of spices and it has been determined that cardamom, one of these spices, protected its antioxidant capacity (100).

6.   Conclusion

As a result of the above-mentioned studies, it is clearly seen that the amount of phenolic compounds and antioxidant activities of all these herbs and spices are different from each other. Many herbs and spices are natural antioxidants

that can be preferred as safer alternatives to synthetic antioxidants used in food production due to their high phenolic compounds and high antioxidant capacity. Developing researches on this subject in order to determine the real effectiveness of these ingredients and to increase the effectiveness of their use in foods is also significant in terms of food safety.

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BIOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF LYCOPENE

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1.   Introduction

Nutraceuticals, which are expressed as a combination/mixture of the words nutrition and pharmaceuticals, are food or food products having health promotion potential and medicinal benefits, and are products offered for consumption in the form of pills, powders or other medicinal drugs (not in food form) (1,2). There is a strong interest in the nutraceutical use of edible foods, fruits and vegetables in the food, cosmetic industries and pharmaceutical (3).

Lycopene, which is a fat-soluble carotenoid (4), provides significant physiological benefits (3). Lycopene, which is synthesized by many plants and microorganisms, is an antioxidant that cannot be synthesized by humans and animals. It is reported that conjugated dienes are active in antioxidant activity (4,5) and that lycopene has a higher antioxidant capacity compared to other carotenoids and especially prevents the risk of prostate cancer formation (6,7).

Lycopene, which is found in excess amount in the structure of fruits and vegetables such as tomatoes, pink guava, watermelon, rosehip, pink grapefruit, strengthens the bonds between cells as well as protecting cells from free radical damage, and improves cell metabolism and lowers cholesterol (3,8).

In addition, lycopene, which has a protective effect in cardiovascular diseases, also slows down the aging process. In this book chapter, the biosynthesis and metabolism of lycopene, as well as its pharmacological and health effects are explained.

2. Lycopene

Carotenoids, which cannot be synthesized by humans or animals, can be synthesized by plants, fungi, bacteria and algae. Carotenoids are a family of pigmented compounds that provide occurrence of different colors to fruits and vegetables from yellow to red (5,9-11). Lycopene, which is one of the carotenoids; is an aliphatic, that is, straight-chain hydrocarbon in the symmetrical plane and a terpene composed of 8 isoprene units. Lycopene contains a total of 13 double bonds, which are 11 conjugated (12-14) and 2 unconjugated, in its structure (Figure 1) (12). Its molecular formula is $C_{40}H_{56}$ and its molecular weight is 536.85 daltons (15,16). Although lycopene is chemically a carotene, it does not have vitamin A activity due to its lack of β-ionone ring structure (12). Lycopene, which is found in all isomeric forms in nature, has both cis and trans isomer forms due to the double bonds in its structure (17).

Lycopene is a red pigment that is found in abundance vegetables in and red fruits such as tomatoes, papaya, pink grapefruit, pink guava and watermelon. This red pigment was named as lycopene by Schunck after it was first discovered by Millardet in 1876. Apart from freshly consumed fruits, it is found in fruit juices, processed and frequently consumed foods such as tomato paste, ketchup, fenugreek (18-22). The name lycopene was derived from the Latin name of tomato, *Solanum lycopersicum*, and is the most common carotenoid found in the human body (23). Lycopene also shows widely distribution in the tissues found in body. It is reported that the amount of lycopene in the tissues is not homogeneous and is found more especially in testis and adrenal gland (24). The reason why these tissues are rich from the point of view lycopene is explained by the fact that they contain a large amount of lipoprotein receptors. Lycopene is also found in the liver, kidney, ovary, lung, colon, breast, and skin (25).
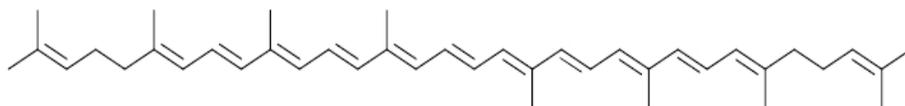
Figure 1. Chemical formula for lycopene

2.1. Biosynthesis of Lycopene

Acetyl CoA, which is the starting material of mevalonic acid, can be synthesized directly from free acetate or it is formed as a result of condensation of pyruvic acid or fatty acids. After the formation of acetoacetyl CoA from acetyl CoA via the thiolase enzyme, HMG-CoA is obtained by adding another acetyl CoA to the reaction via β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase. Mevalonic acid is synthesized from HMG-CoA via HMG-CoA reductase. Mevalonic acid forms mevalonate 5-diphosphate by using a total of 2 molecules of ATP via phosphorus mevalonate kinase in mevalonate 5-phosphate after conversion to mevalonate 5-phosphate with the effect of the mevalonate kinase enzyme. Then, isopentenyl diphosphate (IPP) is formed for the formation of isoprenoid chains with decarboxylation of mevalonate diphosphate via mevalonate diphosphate carboxylase. IPP: The dimethylallyl-PP isomerase enzyme converts IPP to dimethylallyl diphosphate (DMAPP) (11,26,27). Geranyl-PP is formed by condensation of dimethylallyl-PP and IPP via geranyl-PP synthase. In the same way, geranylgeranil-PP is formed by condensation of farnesyl-PP to IPP via farnesyl-PP (11,28,29) and geranylgeranyl-PP synthase with condensation of geranil-PP with IPP via farnesyl-PP synthase. Then, phytoene (3 conjugated double bonds), which is the first product of carotenoid biosynthesis, is formed under condensation of 2 molecules of geranylgeranil-PP (26,30) and phytoene synthase catalysis. After this step, phytofluene (5 conjugated double bonds), the second product, and zeta carotene (7 conjugated double bonds), the third product, are formed by a desaturation reaction catalyzed by the phytoene desaturase enzyme (31-33). After this step, neurosperene (9 conjugated double bonds), the fourth product, and lycopene (11 conjugated double bonds), the last product, are formed via the zeta carotene desaturase enzyme (Figure 2) (31).

Acetyl-CoA + Acetyl-CoA

CoA-SH ← | *Thiolase*

Acetoacetyl-CoA

Acetyl-CoA ⌐ | *HMG-CoA synthase*

β-Hydroxy-β-methyl-glutaryl-CoA (HMG-CoA)

| *HMG-CoA reductase*

Mevalonic acid

ATP ⌐ | *Mevalonate kinase*

Mevalonate-5-phosphate

ATP ⌐ | *Phosphomevalonate kinase (Mevalonate phosphate kinase)*

Mevalonate-5-diphosphate

CO_2 ← | *Mevalonate diphosphate carboxylase*

Isopentenyl diphosphate (IPP) (C5)

| *IPP:DMAPP isomerase*

Dimethylallyl Diphosphate (DMAPP) (C5)

IPP ⌐ | *Geranyl-PP synthase (Dimethylallyl transferase)*

Geranyl-PP (C10)

IPP ⌐ | *Farnesyl-PP synthase (Geranyl transferase)*

Farnesyl-PP (C15)

IPP ⌐ | *Geranylgeranyl-PP synthase (Farnesyl transferase)*

Geranylgeranyl-PP (C20)

Geranylgeranyl-PP ⌐ | *Phytoene synthase*

Phytoene (C40)

| *Phytoene desaturase*

Phytofluene

| *Phytoene desaturase*

Zeta-carotene

| *Zeta-carotene desaturase*

Neurosporene

| *Zeta-carotene desaturase*

Lycopene (C40)

Figure 2. Biosynthesis of lycopene

3. Pharmacological Properties and Effect on Health

Lycopene, which is used as food colorant, feed additive and nutraceutical for pharmaceutical purposes is also used for prophylactic purposes in cancer and neurodegenerative diseases, as well as as an antihypertensive in cardiovascular diseases due to the inhibition of angiotensin converting enzyme (3,34-36).

Oxidative stress occurs due to the increase of reactive oxygen species (ROS), which are formed as the end product of electron transport chains and redox cycle. Increasing ROS or its metabolites damage cell components such as lipid, protein, and DNA in the body. Lycopene, which is an antioxidant in the body, has functions such as free nitrogen dioxide of singlet oxygen, scavenging of thiol and sulfonyl radicals. Singlet deoxygenating power of lycopene is 2 times stronger than β-carotene and 10 times stronger than α-tocopherol. Due to this feature, the studies were that it has a therapeutic effect in diseases and cancers of organs such as the urinary bladder, breast and large intestine. In a study conducted (37), lycopene was shown to have therapeutic efficacy by reducing oxidative stress in gastric ulcer induced by indomethacin in rats. In another study conducted, it was reported that likopenin shows antioxidant activity by lowering thiobarbituric acid reactive substance (TBARS), protein carbonyl content and myeloperoxidase levels of lipid peroxidation product as it increases the antioxidant level of lycopene in cardiotoxicity in rats (38). In addition, Kim et al. (39) reported that there is an inverse correlation between lycopene levels and C-reactive protein levels and that dose-dependent lycopene supplementation will be able to play a role in endothelial functions by reducing oxidative stress in a study they conducted. Lycopene prevents inflammation by suppressing prostacyclin, prostaglandin, thromboxane, and leukotriene synthesis by regulating lipoxygenase and cyclooxygenase enzymes that cause inflammation (40).

Cancer takes part among the most important reasons causing to death in the world (41). It is known that nutrition has an important role in the prevention of chronic diseases. They reported that the risk of some cancer types such as colon cancer is lower in animals fed with foods containing high lycopene such as tomatoes and tomato products (42). Lycopene was also shown that it induces apoptosis and has the potential to be used as a chemotherapeutic agent in numerous in vitro studies contected by using cancer cells from various tissues (43). It was observed that feeding with a diet containing a combination of vitamin E, selenium and lycopene significantly reduces prostate cancer and

liver metastasis compared to taking these substances separately (44). Beneficial effects of dietary supplementation of lycopene in the treatment of prostate cancer were observed. In addition to this, it was revealed that lycopene consumption reduces the total cholesterol concentration in the human body (45).

In a study investigating the effect of lycopene in rats with experimental diabetes, it was reported that lycopene administration has positive effects on the immune system in diabetic rats (46). Moreover, Kaya et al. (47) reported that lycopene administration in the group administered with lycopene+diethylnitrosamine (DEN) provides improvement for the biochemical parameters of hepatotoxicity in both blood and liver tissue compared to the group given only DEN in another study they conducted in rats. In addition, in a study conducted in rats (48), it was reported that lycopene, which is a natural antioxidant and anticarcinogenic, will be able to have a protective effect against flutamide-induced hepatotoxicity in the liver of rats. In addition, it was reported that β-carotene, which is a carotenoid such as lycopene, will be able to have a protective effect against hepatotoxicity in rabbits given DEN when compared to the group given β-carotene + DEN (49,50). It was reported that the damage caused by 3-NPA is reduced due to the antioxidant properties of astaxanthin and it will be able to have a healing effect in the group given astaxanthin in ovarian damage induced by 3-nitropropionic acid (3-NPA) in rats (51).

4. Conclusion

As a result, it was revealed in many studies that lycopene, which has many physiological properties, has antioxidant, antibacterial and anticarcinogenic properties. For these reasons, it is recommended to consume foods rich in lycopene, such as tomatoes and tomato products.

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MUMIE: AN APPROACH ON POTENTIAL BIOCHEMICAL PROPERTIES AND HEALTH ACTIVITY

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1.   Introduction

Mumie, also known as salajit, shilajatu, moomiyo, or mummiyo, is formed as a pale brown to blackish-brown exudation a multicomponent naturally occurring mineral of variable consistencies, derived from rocks of diverse structures at altitudes approximately 1 and 5 km (1). Molecularly, mumie consists mainly of humic substances, which are the result of degradation of plants by several microorganisms, especially fungi (2). It is generally stated that during the hot summer months (May-July), mumie flows through cracks and spreads over the rock surface. This mineral of variable consistency is considered to be composed of different materials such as fossilized marine invertebrates, resins, and plants. But its composition varies widely, and its exact origin is still a matter of debate (1,3,4). Mumie, a paleo-humus, is an unusual and intriguing medicinal substance. Mumie is located in different mountain regions of the world (1,4,5). Mumie is referred to as rasayana in Ayurveda and is considered to prevent ailments and improve the quality of life. The health benefits of mumie

have been shown to differ from region to region, depending on the place from which it was extracted (3,6,7). Traditionally used for diabetes, anemia, ulcers, bronchial asthma, gastrointestinal infections, liver diseases, geriatric problems, sexual dysfunctions, rejuvenating, aphrodisiac effects and wound healing (1,5,8).

This chapter focuses on the potential biochemical propertie and health efficacy of mumie, a paleohumus and organic compound. This review also aimed to summarize the human, animal, and in vitro research that supports these health claims, with emphasis on well-controlled studies published over the past years and recently.

2. General Characteristics of Mumie

2.1. Formation and Origin of Mumie

Mumie consists of paleohumus (around 80-85%) and organic compounds derived from vegetation (mainly bryophytes such as Euphorbia and Trifolium (clover) plants and lichen) fossils (herbo-mineral, marine animal origindead/ fossil invertebrates). These organic compounds are formed by being compressed in rock layers for many years and undergoing metamorphosis under the influence of high temperature and pressure (3,9-14). In addition, different weather conditions related to summer and winter temperatures, sunshine duration and precipitation are important factors in the formation of mumie. (14,15). During the hot summer months, the temperature of the mountains rises and the mummy becomes less viscous and flows out through the layers of rock (16,17,18). Organic exudates are found in some mountainous regions of the world in the form of rock exudates of up to 500 kg, embedded in the rocks or spread on the surface, on the walls of caves at an altitude of about 1000 and 5000 m (3,9). Mumie' s colour may vary from blackish to brown (Figure.1) (a pale-brown to blackish-brown). (14,15). Mumie has a porous or smooth surface, irregular fragments, tarry, solid and elastic character (19-21). It is bitter in taste and its smell resembles pungent (characteristic balsamic odor) (17,18).

Figure 1. Mumie in raw (A) and processed (B, C, D) form.

Mumie, especially in Central Asia (Himalaya, Pamir and Altai), Kashmir, Afghanistan, Nepal, Bhutan, Pakistan, China, Tibet, Yemen, parts of the former USSR (Kyrgyzstan, Tajikistan, Uzbekistan, Kazahstan) and nearby regions (Ural, Baykal, Sayan, Caucasus and Altai) in mountainous areas (3,6,7,15,22). Mumie is widely found in the Himalayas region starting from Arunachal Pradesh in the East to Kashmir in the West. It is also spread in other countries, such as Afghanistan (Hindukush), CIS (Tien Shan, Ural), Tsao-Shing, Australia, Mongolia, China, Bhutan, Nepal, Pakistan, Tajakistan (Zarafshan) and Tibet-Himalayan belt (3,6,23,24). It is also available in these countries as it was imported from Yemen or India to Japan, Algeria and Saudi Arabia (15,25). Mumie is used in the form of an aqueous extract for therapeutic applications such as immunostimulants and anabolic food additives (12). The health effectiveness of mumie varies from region to region, depending on the area from which it is supplied (3,7). In the literature, this difference has been attributed to the active ingredient content depending on weather conditions and decomposition of many plant species (3). Despite the fact that the yield of mumie in nature is very low and specific in a particular geographical areas, the bioeconomy of this natural product is maintained automatically (26).

2.2. Chemical Constituents of Mumie

Samples taken from their regions of existence have similar physical properties and qualitative chemical composition but they differ in the ratio of individual

components (27). It is considered that the debris of several plants growing in those areas are responsible for the formation of mumie. Its content was found composed of humus and organic matter (60-80%), mineral matter (20-40%) and ~5% trace elements. These main chemical components are humic acid (HA), fulvic acid (FA), di-benzo-alphapyrones and related metabolites, tannic acid, benzoic acid, benzoates, oxygenated biophenyls, oxygenated benzocumarins, several phenolic compounds, amino acids, triterpenes, small peptides, some phenolic lipids, hippuric acid, free fatty acids, essential oils, organic acid, adipic, succinic, citric, oxalic, tartaric, sichthyol, ellagic acid, sterol, aromatic carboxylic acid, uronic acids, phenolic glucosides, gums, albuminoids, resin, waxes, vegetable matter, glycosides, moisture, anti-oxidant agents, high concentrations of vitamin (A, B1, B2, C esters) and enzymes (4,6,19,22,28-32). The rate of the presence of some of these substances is as follows: 14-20% humidity; 18-20% minerals; 13-17% proteins (with marked-α-amylase activity); 4-4.5% lipids; 3.3-6.5% steroids; 18-20% nitrogen-free compounds; 1.5-2% carbohydrates; and 0.05-0.08% alkaloids, and a number of amino acids (15). Also, modern chemical analyses identified six new compounds named as shilajityl acetate, shilajitol, shilacatechol, shilaxanthone, shilanthranil and naphsilajitone along with pyrocatechol and their stereostructures (14). More recently, five new diterpenoids, referred to as mumic acids A-E, have been isolated and structurally characterized using spectroscopic data and chemical derivatization (33). Some studies found that it contains more than 84 minerals, including copper, silver, zinc, iron, magnesium, sulfur, iron, chloride, phosphorous, iodine, calcium, potassium, nitrogen, silica and lead in their ionic forms (29,34). It is found that the differences in mumie color were generally due to the differences in the content of minerals such as iron, copper and silver (1,3). Humin, humic acid and fulvic acids are defined as humic substances and can be dissolved in water with different pH values. FA is soluble in water at all pH conditions, humic acid is soluble in water at alkaline pH conditions, while humin is insoluble in water at any pH condition. Humic acid has a molecular weight of 5-10 kDa and FA has a molecular weight of 800 kDa. It has been reported that the main therapeutic and curative effect of mumie in terms of health is due to the presence of bioactive dibenzo-a-pyrones together with humic and FA. **(Figure 2)**. It has been observed that these bioactive substances are well absorbed from the intestines and are eliminated from the body within a few hours. Today, many medicinal benefits of mumie are dedicated to these properties (6,30,35).

Antioxidant activity	The effects in anti-muscular and cardiovascular system
Antidiabetic activity	Overcome high-altitude-related problems
Antiviral activity	Effects of mumio on osteoblastic differentiation and bone regeneration
Anti-ulcerogenic	Learning augmentation and memory enhancement activty
Antistress activity	Effect on neurodegenerative diseases
Anti-inflammatory and analgesic activity	Immunomodulatory activity
The effects profertility reproductive and hormonal actions	

Figure 2. Mumie's main ingredients and potential biochemical health benefits.

2.3. Synonyms and Meaning of Mumie

Charaka Samhita describes mumie as "stones of metal like gold" while Sushruta Samhita describes it as a "a gelatinous substance." (8,36). Mumie (botanical name: Asphaltum), also known as mineral pitch and it has various names (also

known as Çilájatu, silaras adrija, girija, asphalt, mountain sweat, mountain oil, rock juice, vegetable asphalt, momiai, mummy or mineral pitch) (8,29,36,37). In Sanskrit, it is called Silajit or Silaras, adrija, girija (all meanings derived from rock). These terms meaning "conqueror of mountains and destroyer of weakness" and 'winner of rock' (7,38). Also, there are several other terms for mumie such as dathuras, dathusara, shiladhatu, etc. have been used in ancient medical texts. The word dhatu was used as a synonym of mumie simply to emphasize its capability as rasayana, which increases the activity of the sapthadhatus of the body (39). Mumie, also known in northern India (In Hindi, Gujarati and Marathi, it is called Silajita, Shilajit) as salajit, shilajatu, mimie or mummuyo. In Tibet, Afghanistan and northern Chile it is called the Andean mumie (30,40). The name ''mumie, hajar-musa, Hajarul-musa or mumia'' was devised by the Arabs and in ancient Egypt (12-14). It is also called, Momio in Persian, myemu in Russian and middle Asia, mumie in German. In English, it is called asphalt, mineral pitch, Jews pitch, Mumiyo, Mineral wax, or Ozokerite. In Bengali, it is called Silajatu (7,8,38,41). Depending on the abundance of metals present in the rocks, it has been classified by the texts into six types namely, Suvarna (gold), Rajat (silver), Tamra (copper), Lauha (iron), Naag (lead) and Vanga (tin). The Loha type is commonly found and used therapeutically (42).

3.   Biochemical Effects of Mumie

Mumie has an expected broad biochemical activities due to its contents of fulvic acid and other antioxidant materials (3,4,43). In diabetic rats, three doses of mumie were found to produce a significant reduction in blood glucose levels and also produce beneficial effects on the lipid profile (44). The results of another study suggest that administration of processed shilajit (PS) along with insulin would potentiate the insulin-induced hypoglycaemia, and chronic administration of the dose of PS would inhibit the development of STZ-induced diabetes (45). Mumie samples from different mountainous regions such as Yemen (Al-Jouf and Rayma), Russia (Tien-Shan) and India (Kumoan) were studied in rats. Plasma lipids showed a significant lower level of total plasma cholesterol in all mumie treated groups when compared with saline pretreated group. Furthermore, the mumie-treated group from Al-Jouf and Rayma showed a significantly lower total plasma cholesterol level than the ranitidine-treated group. Additionally, all pretreated groups (except Russian) showed a significant increase in plasma HDL-cholesterol level than saline pretreated group, while all pretreated groups (except Russian) showed a significant increase in plasma HDL-cholesterol level

than ranitidine pretreated group. Results showed a significant decrease in plasma triglycerides level in all treated groups than saline pretreated group, while all pretreated groups (except Indian and Russian) showed significant decrease in plasma triglycerides level than ranitidine pretreated group (43). In a different study, administered 2000 mg of processed mumie or placebo per day for 45 days to human subjects. Twenty subjects received mumie and 10 subjects received placebo. Significant decreases in serum cholesterol, low density lipoprotein, very low density lipoprotein, and triglycerides were observed in response to mumie as compared to the placebo group. High density lipoprotein also increased in mumie-treated subjects (46). Diabetes mellitus was induced by the administration of streptozotocin (STZ, 45 mg/kg, S.C. on 2 consecutive days) in male Wistar rats. Hyperglycaemia leves of pancreatic islet cells assessed on days 7, 14, 21 and 28, following STZ administration. In two other groups, mumie (50 and 100 mg/kg, p.0.) administered concurrently for 28 days. Mumie (50 and 100 mg/kg, p.0) was found to have no self-detectable effect on blood glucose levels in normal rats, but at higher dose from day 14 onwards attenuated the hyperglycemic response of STZ. The findings support the postulate that mumie can prevent maturity onset diabetes mellitus (47). 28 male subjects received treated mumie at a dose of 100 mg twice daily for 90 days. Small but significant reductions in fasting blood glucose and creatinine levels were observed in subjects treated with mumie (48). Raju et al. (2012) administered processed mumie at a dose of 250 mg twice daily for 90 days in safe studies involving 43 healthy human volunteers. They did not detect any changes in kidney or liver function tests. In addition, they determined that mumie treatment decreased fasting blood sugar, uric acid and erythrocyte sedimentation rate, while increasing hemoglobin and platelet count (34). The hepatoprotective activity of mumie (Asphaltum punjabinum) and its effects on blood factors were evaluated in-vivo and in-vitro by alcohol-induced inhibition of the Wistar rat. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), Total Bilirubin (TBIL) and Direct Bilirubin (DBIL) were examined in the serum of rats. In the study, demonstrated the Asphaltum punjabinum has potent hepatoprotective activity against alcohol induced hepatic damage in experimental animals. Also, the histopathological observations supported the biochemical evidences of hepato protection (49). A similar study evaluated the effects of mumie on liver damage. Forty male Wistar rats were fed a high-fat diet to establish models of fatty liver. It was determined that mumie treatment significantly reduced aspartate aminotransferase (AST)

and alanine aminotransferase (ALT,) triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), glucose and liver glutathione peroxidase (GPx), superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, liver weight, and steatosis values. Instead, there was an increase in HDL compared to the vehicle group. In addition, mumie treatment improved the side effects of feeding a highfat diet-induced histopathological changes in the liver compared to the vehicle group (50).

4. Pharmaceutical Effects of Mumie

The physiological and pharmacological effects of mumie are attributed to the DBPs, DBP chromoproteins (DBPs conjugated to proteins), fulvic acid, and various polymeric forms of fulvic acid (1,12, 34,46). Since in mumie is usually present more than 10% as the main components along with free and conjugated dibenzo-α-pyrons (DBPs; Urolitins), DBP chromoproteins and more than 40 minerals, Health Benefits High-quality products used in dietary supplements are standardized to contain at least 50% fulvic acids and equivalents (polymers and related structures) along with dibenzoa-pyrones (DBPs) and DBP chromoproteins (34, 51). High-quality products used in dietary supplements should have a water-soluble extraction value greater than 80% (19). In the literature, health benefits have been concentrated in this regard, as the primary effects of mumie stem from the ability of fulvic acid components to chelate product-associated minerals and facilitate cellular penetration (3,30). Generally, mumie has an expected broad biochemical and pharmacological activities due to its contents of fulvic acid and other antioxidant materials. Fulvic Acids (FA) has been taken orally as a therapy for gastritis, stomach ulcers and colitis (3,4,43). Due to the low overall mineral content of mumie, it is doubtful that a significant amount of the mineral is absorbed and penetrates cells, as the vast majority of minerals present occur in extremely small amounts at given doses. From a general point of view, in a typical mumie dose of 200 mg, the total mineral content will be 2-3 mg, with about 90% being potassium, calcium, and magnesium. If we take this into account, the typical daily recommended intake for calcium is 1000-1200 mg, whereas the daily values for magnesium and potassium are 400 mg and 3000 mg, respectively. Because mumie content is variable and its overall complex nature, the processing required to prepare the final product, and the difficulty in standardizing the finished product, counterfeiting and adulteration are major problems (12). As a consequence, consumers are cautioned to use products from known and reputable manufacturers and suppliers. Mumie

is processed by several drug manufactures and marketed in capsule form for human consumption (52).

5. Traditional and Modern Therapeutic Uses

Mumie has been used in different forms for over 3000 years as a rejuvenator and adaptogen under indigenous systems of medicine such as Ayurveda, Siddha, and Unani (17,18,53). Like mumie in ancient Indian Ayurvedic medicine, the rasayana compound has two important properties: first one, to increase physical strength and second, to promote human Health and antiaging (6). Also traditionally has been used as in folk medicine blood sugar stabilization, urinary tract rejuvenation, enhanced brain functioning potency, kidney rejuvenation, immune system strengthening, arthritis, urinary, immune, digestive, cardiac, anemia, emesis, nervous systems, hypertension, ulcers, bronchial asthma, gastrointestinal infections, liver diseases, geriatric problems, sexual dysfunctions, rejuvenating, aphrodisiac effects, wound healing as well as for treating many other conditions (1,5-8,36,54,55). In many countries mumie has been used for in treatment of genitourinary diseases, diabetes, digestive disorders, nervous diseases, tuberculosis, chronic bronchitis, asthma, jaundice, anemia, eczema, bone fractures, osteoporosis, kidney stones, edema, spondylitis, hemorrhoids, injured muscles, bone fractures, and diseases such as osteoporosis and other diseases; it is also used as a rejuvenator and an internal antiseptic (3,4,29,56).

In modern systematic research has scientifically validated a number of medicinal properties significant such as anti-inflammatory activity, free radical elimination functions (antioxidant), anxiolytic effects, anti-fungal, anti-ulcerogenic, anxiolytic activity, anti-allergic, analgesic, anti-diabetic, memory enhancer, chemoprotectant and immunomodulator, thereby advocating mumie as a truly panacea in traditional medicine (6,29,47,51,56-60) It has also been noted to have anti-oxidative, spermatogenic and ovogenic effects (48,61). In addition to these in ancient Egypt, this wonderful resin was used for embalming mummies (3,12-14). The safety of the mumie is well studied and is generally regarded as a safe substance (51,62). For therapeutic purposes in different studies it is administered in the form of an aqueous extract to activate phagocytosis and cytokine release by murine peritoneal macrophages, stimulate osteoblastic differentiation of mesenchymal stem cells, induce the proliferation of lymphocytes in the cortical thymus layer and increase migration of these cells into thymusdependent zones of the lymph nodes and spleen (63,64). In the aqueous extract of mumie humus comprise fulvic acid (FA) as the primary

organic substance endowed with for many biological and medicinal properties effective in the treatment of disorders including gastritis, diarrhea, stomach ulcers, dysentery, colitis and diabetes mellitus and stimulate neutrophil and lymphocyte immune function (3,4,12,58). In some states, it is have using as a dietary supplement, either as a stand-alone product or in combination with other ingredients (52).

5.   PRECLINICAL RESEARCH

5.1. *Antioxidant activity*

Anti-oxidant are known as "free radical scavenging activators" which neutralizes free radicals that are generated in the body and prevent damage to the cell proteins, lipids and carbohydrates. Various studies indicate mumie a natural mineral that it possesses antioxidant properties (3,54,56,60). Antioxidant properties of mumie extract have been attributed to the presence of dibenzo-α-pyrones and fulvic acid (4). It is likely that the curative properties attributable to mumie are provided by the significant levels of fulvic acids that mumie contains, considering that fulvic acid is known by its strong antioxidant actions (65).

Preclinical studies in adult male Wistar rats revealed that processed mumie provides complete protection to methyl methacrylate against hydroxyl radical-induced polymerization. Mumie (20 and 50 mg/kg/day, i.p., for 21 days) has been reported to a dose-related increase SOD, catalase (CAT) and GPx activities in the corpora striatum and frontal cortex of rats (60). The effect of standardized processed mumie was evaluated in a rat model of chronic fatigue syndrome (CFS). Mumie has been shown to reverse CFS-induced mitochondrial oxidative stress in terms of nitric oxide (NO) concentration and lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase activities (66). The effect of diet supplemented with mumie, on the antioxidant activity, immune response, and disease resistance in freshwater prawn, Macrobrachium rosenbergii (de Man) against Aeromonas hydrophila is researched. According to the results, it was stated that the diet enriched with mumie at 2 g kg^{-1} or 4 g kg^{-1} significantly increased glutathione peroxidase (GPx), phenoloxidase (PO), superoxide dismutase (SOD), glutathione reductase (GR) activities (67). In a 6-week feeding study in chicks, Mumie was shown to prevent lead-induced oxidative stress (68).

In the a clinical study with mumie to show its effect on antioxidant activity in diabetic subjects, 61 diabetic subjects of either sex, aged 31-70 years were

administered mumie as two capsules (500mg each; Dabur, India) twice daily for 30 days. Subjects treated with mumie exhibited a significant decrease in values malondialdehyde compared with their higher pre-treatment values, while catalase values in diabetic subjects were reported to increase significantly after treatment with mumie (69).

Unfortunately mumie lacks systematic documentation and well-established clinical trials on its antioxidative actions in humans, and it is expected that considering the reported benefits evidenced fromtrials will be obtained in the near future (5).

Various in vitro studies have been conducted to obtain information regarding possible mechanisms of action of mumie (70). The antioxidant activities of 3-hydroxy-DBPs and the 3,8-dihydroxy-DBPs, which are believed to be active constituents of mumie, were demonstrated in vitro using five free radical scavenging assays (71). In another recent in vitro study, it is noted that, Shilajatu potentiates the antioxidant activity. In study, the rat liver homogenate treated Shilajatu have showed less degree of carbon tetra cloride (CCl_4) induced lipid peroxidation. CCl_4 induces oxidative stress by free radical mechanism. Thus, they showed that oxidative stress caused by CCl_4 concentration in homogenate treated with Shilajatu 1%, 2% and 5% and malondialdehyde (MDA) level, which is a marker of oxidative stress, decreased significantly (72). In a recent study, the antioxidant, hepatoprotective activity of mumie (Asphaltum punjabinum) was investigated and its effects on blood factors were evaluated in-vivo and in-vitro by inhibition of alcohol-induced Wistar rat. As a result, while the oxidant molecule MDA was prevented in liver tissues, the antioxidant enzymes super oxide dismutase (SOD), reduced glutathione (GSH) and catalase increased. Also, DPPH showed strong activities on the superoxide anion. Increasing SOD level and decreasing MDA level further strengthen hepatoprotective observations. In the study, it was shown that Asphaltum punjabinum has strong antioxidant and hepatoprotective activity against alcohol-induced liver damage in experimental animals (49).

5.2. *Antidiabetic Activity*

Mumie is mentioned under rasayana category in Charaka Samhita, the oldest text of Ayurvedic system of medicine. Charaka has stated that mumie can be used in several diseases by altering the anupana (vehicle) and adjuvant in combination with several drugs. Sushruta has described mumie in madhumeha chikitsa (diabetes mellitus). In his text, purified mumie is advocated in madhumeha

along with the decoction of Shorea robusta group of plants. It has also been previously stated that Mumie is given with milk to control diabetes plants (5). Some studies have examined the antidiabetic effects of mumie in animals. In one study, they observed that a processed mumie (1.0 mg/kg subcutaneously) prevented streptozotoxin-induced diabetes in rats and mumie potentiated the hypoglycemic effect of insülin (45). In another study, oral administration of a 50 mg/kg and 100 mg/kg process and standardized mumie was shown to reduce streptozotocin-induced diabetes in rats. This experiment supports the earlier writing of Ayurveda that mumie can prevent maturity onset diabetes mellitus (47). Trivedi et al studied the effect of mumie (a herbomineral preparation) on blood glucose and lipid profile in euglycemic and alloxan-induced diabetic rats and its effects in combination with conventional antidiabetic drugs. Diabetes was induced in albino rats by administration of a single dose of alloxan monohydrate 5% (125 mg/kg, i.p.). Effects of three different doses of mumie (50, 100 and 200 mg/kg/day, orally), for 4 weeks were studied on blood glucose and lipid profile. In the diabetic rats, all the three doses of mumie produced a significant reduction in blood glucose levels and also produced beneficial effects on the lipid profile. Also, the maximum effect have observed with the 100 mg/kg/day dose of mumie. As a result, mumie was hypothesized to be effective in controlling blood sugar levels and improving the lipid profile. It has also been stated that mumie is worth clinical trial as monotherapy or in combination with other antidiabetic agents, as it can provide good glycemic control in animals (44).

5.3. *Antiviral Activity*

Fast evolution and mutation of viruses set forward a need for new antiviral agents suitable for treatment of drug-resistant infections (73). Moreover, the pandemy of COVID-19, which hit already more than 5 million people globally, shows how limited a pool of antiviral drugs (74). In this respect, the natural supramolecular systems of biologically active compounds might be of particular value. Humic substances (HS) have been known for a long time for their antiviral activity (75). It is stated that the biological activity of mumie is usually due to humic substances (HS) and that these humic substances provide antiviral activity to mumie (12). In addition, the most hydrophilic fraction of fulvic acid (FA) had the least activity. Structure-activity analysis indicated that there is a direct relationship between the antiviral activity and the ratio of aromatic and aliphatic structures of different humic fractions used, and an inverse relationship with their carboxylic and total acidity. In study on the anti-

HIV activity of HS isolated from peloids (fresh water bottom sediments), ıt was observed that the strongest inhibiting activity was characteristic of the most hydrophobic fractions of humic acids and hymatomelanic acids (HMA) (76). In another study, mumie exhibited a dose-dependent inhibitory activity against HSV1, HSV2, HCMV, and RSV infectivity in vitro. It has been determined that humic acid, a component of Mumie, exhibits the same spectrum of activity. Partial virus inactivation and interaction with virus attachment have both been hypothesized to contribute to the antiviral activity of mumie (77). In a similar study, the anti-HIV activity of well-characterized HS isolated from coal, peat and peloids was evaluated and compared with the water-soluble organic matter (OM) isolated from different mumie samples. As potential carriers of antiviral activity they have determined aromatic structures with alkyl substituents, terpenoids, N-containing analogs of typical flavonoids, and azapodophyllotoxins. Besides, the conclusion have made that the typical humic materials and mumie differ greatly in molecular composition, and the humic materials have substantial preferences as a natural source of antiviral agents as compared to mumie (78).

5.4. *Learning Augmentation and Memory Enhancement Activty*

The memory enhancing feature of mumie is also noteworthy (30). In the first of the previous studies, the effect of mumie was investigated for putative nanotropic and anxiolytic activity in Charles Foster strain albino rats. The results of these studies indicated that mumie had significant nanotropic and anxiolytic activities. The biochemical studies carried out for the level of monoamines indicated that acute treatment with mumie had an insignificant effect on rat brain monoamines and monoamine metabolite levels. The observed neurochemical studies on mumie indicate a decrease in rat brain 5-hydroxytryptamine turnover, associated with an increase in dopaminergic activity leading to an increase in memory and anxiolytic activity in albino rats (59). In the second, was carried out to test the validity of use of mumie as an Ayurvedic medha rasayana (enhancer of memory and learning) in albino rats. Processed mumie, native mumie and a preparation consisting of a mixture of ethyl acetate extractive and fulvic acids obtained from processed Mumie were evaluated in an active avoidance, elevated plus-maze and open field behavior paradigms. It was found that processed mumie and its active constituents (total ethyl acetate fraction and fulvic acids) significantly increased the learning acquisition and memory retention in old albino rats (38).

5.5. *Anti-ulcerogenic*

It has been stated that mumie is an agent with antiulcerogenic activities and can be used safely in clinical practice with this unique feature (3). Previous studies support the antiulcerogenic activity of mumie. Thus, It was found that mumie increased the carbohydrate/protein ratio and decreased the gastric ulcer index, indicating an increased mucus barrier Also, mumie was stated to be beneficial in cases of digestive disorders, wound healing, gastric ulcer and gastrointestinal system (29,58). At the same time, Fulvic Acid, one of the active ingredients in mumie, have been taken orally for the treatment of gastritis, gastric ulcer and colitis (3,4). Similar experimental a study, suggested that the antiulcerogenic effect of fulvic acids and biphenyls isolated from mumie was due to protection of the gastrointestinal mucosa with less shedding of mucosal cells (1). El-Sayed et al(2012) evaluated the effects and mechanisms involved in the anti-ulcer activities of different natural mumie samples. They pre-treated with mumie samples (600 mg kg-1, p.o.) 14 days before ulcer induction. In conclusion, they found that mumie samples inhibited both ulcer score and lesion area with greater percentages. In addition, they determined that the studied mumie samples showed anti-ulcer activities against induced gastric ulcer (43).

5.6. *Anti-inflammatory and Analgesic Activity*

The 200 mg/kg IP dose of mumie has been shown to exhibit significant analgesic activity compared to controls using the rat tail flick method (56). Mumie extract has been found to facilitate the process of wound cleansing, granulation and epithelialization from necrotic tissues due to its anti-inflammatory effect and reduces wound healing time (79). Mumie has been found to have significant (to 77%) antiinflammatory effect in carrageenan-induced acute pedal oedema, granuloma pouch and adjuvant-induced arthritis in rats (29). It is assumed that these anti-inflammatory properties of mumie are due to the presence of fulvic acids, benzoic acid, 4-methoxy-6-carbomethoxybiphenyl and tirucallan-type triterpenoids in its composition (22,58,80). The anti-inflammatory and antiarthritic effects of mumie were studied in moderately arthritic dogs in a randomized placebo-controlled double-blind study. Ten animals received either 500 mg of mumie twice daily or placebo for 5 months. Animals receiving mumie had a significant reduction in pain at day 60 and a maximum reduction in pain at day 150 (81). Mumie

has also been shown to attenuate acetic acid and formalin-induced writhing in mice, thus demonstrating its antiinflammatory activity. The results have showed a significant decrease in pain intensity for all mice receiving doses of mumijo extract during a 1-h formalin test when compared with the distilled water group. A dose-dependent increase in the analgesic effects of mumie was demonstrated at doses of 0.75, 7.5, and 75 mg/kg. In a writhing test, a significant inhibition of the pain response induced by acetic acid also occurred in all 4 mumijo-administered groups as opposed to the group receiving distilled water. No significant differences were observed between 75 and 750 mg/kg mumie and up to 4 mg morphine or 30 mg sodium diclofenac, which were used as positive controls, but in the writhing test, the maximum dose were showed a more effective analgesic action (82).

5.7. *Immunomodulatory Activity*

It is stated that dose and time dependent exposures of mumie of immune systems exhibit different biological manifestations. The key active molecules of mumie responsible for immunomodulatory activity are bis-dibenzo-alpha-pyrone ferrate complex structures, fulvic acids and the DCPs (1,83). In an effective study, which suggest that mumie enhances the lytic potential of polymorphonuclear leukocytes, the administration of a 200-600 mg/dose of mumie to mice resulted in significant morphological and phagocytotic changes in peritoneal macrophages, demonstrating its immunomodulatory capabilities (83). Moreover, mumie at an intraperitoneal dose of 25 and 50 mg/kg for 5 days significantly lowered the levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid and raised the levels of dopamine, noradrenaline, and its metabolites in rat brain. These changes in neurotransmitter levels were similar to those seen in cases of increased humoral (immune) activity (6). Besides, pure mumie was found to supplement the lytic potential of activated lymphocytes and produced T-cell-mediated cytotoxicity. Both fulvic acids containing small amounts of dibenzo-alpha-pyrones (DBPs) and 3,8-dihydroxydibenzo-alpha-pyrones at intraperitoneal doses of 400 µg/mL have been shown to inhibit the proliferation of Ehrlich ascites tumor cells. Ultimately, the immune regulation of cells was thought to be mediated by mumie components. (41). In an in vitro study, KU812 cells incubated with fulvic acid affected the expression of genes involved in signal transduction, cytokine-cytokine receptor interaction, and immune response pathways, as well as cell adhesion molecule and IgE receptor b subunit responses (84).

5.8. Antistress Activity

Mumie collected from India, Nepal, Pakistan and Russia and organic constituents isolated from them were studied for their antistress effect in albino mice (22). In context, anti-anxiety activity and anti-stress effects of mumie has a role in healing of gastric ulcer as stated by Frawley and Lad (2004) whom indicate that mumie has significant anxiolytic and anti-stress activity as reported by). Mumie is truly a remarkable substance with a long history of human usage for healing and should be subjected to further investigations (85).

5.9. Effect on Neurodegenerative Diseases

New research shows that fulvic acid is an antiaggregation factor of the tau protein in vitro, reflecting it as a potential Alzheimer's disease molecule. Considering the actions of fulvic acid in preventing tau self-aggregation into pathological filaments, this compound appears to be of interest for prevention of Alzheimer's disease (40). In the study examining the systemic and cholinergic effects of mumie in the rat brain, the data suggest that mumie affect preferentially events in the cortical and basal forebrain cholinergic signal transduction cascade (86). In a study, the interaction of prion protein with fulvic acid and its inhibitory effect on the content of β-sheet structure and the formation of protein aggregates has been described in clearly (87). The effects of fulvic acid on heparin-induced tau aggregation were investigated in an in vitro study. In the study, inhibition of tau aggregation in the presence of fulvic acid was evaluated by three complementary techniques: thioflavin T fluorescent (ThT) aggregation analysis, atomic force microscopy (AFM) and electron microscopy (EM). In conclusion, the observation of aggregates formed by AFM and EM concluded that fulvic acid inhibited heparin-induced tau aggregation in vitro. On the other hand, fulvic acid promoted the disassembly of tau-preformed fibrils. Thus, fulvic acid has been presented as a novel approach to develop treatments for AD based on natural products (40). Thus, fulvic acid, the main active principle, blocks tau self-aggregation, opening an avenue toward the study of Alzheimer's therapy. Its main medical application, mumie, is now recommended as a dietary supplement for its cognition-beneficial actions and potentially to prevent Alzheimer's disease (30).

5.10. The Effects in Anti-muscular and Cardiovascular System

It has been reported that mumie has central nervous system calming components and relieves skeletal-muscular pain (88). Studies of anabolic properties made it

possible to use mumie in for increasing strength and muscle mass as well as for its recuperative Powers (10). In one study, Bhattacharyya et al. (2009b) used a forced swimming task in mice to examine the effects of PrimaVie® mumie supplementation on indices of mitochondrial function including post-exercise muscle ATP concentration and adenylate energy charge. Oral PrimaVie® mumie supplementation (30 mg·kg⁻¹ of body weight for 4 days) resulted in a significantly greater (p<0.001) post-exercise ATP concentration of 0.49 ±0.05 μmol·g⁻¹ of muscle compared to 0.25±0.05 μmol·g⁻¹ for the swimming only group without PrimaVie® mumie supplementation. As a result, hypothesized that the augmented mitochondrial function, improved energy status, and upregulated (89). Keller et al (2016), examined the effects of 8 weeks of mumie supplementation at 250 mg·d⁻¹ (low dose) and 500 mg·d⁻¹ (high dose) versus placebo on maximal voluntary isometric contraction (MVIC) strength, concentric peak torque, fatigue-induced percent decline in strength, and serum hydroxyproline (HYP). The results of the study showed that 8 weeks of PrimaVie® Mumie supplementation at 500 mg·d⁻¹ promoted the retention of maximal muscular strength following the fatiguing protocol and decreased baseline HYP. Thus, PrimaVie® mumie supplementation at 500 mg·d⁻¹ elicited favorable muscle and connective tissue adaptations (90). In a previous similar study, reported that consuming 500 mg·d⁻¹ of PrimaVie® mumie for 8 weeks upregulated extracellular matrix (ECM)-related gene expression, which promotes collagen and connective tissue integrity (91). Thus, mumie supplementation may have a beneficial effect on exercise performance by enhancing fatigue-related metabolic characteristics and, potentially, increasing muscle mass and strength (90).

Joukar et al. (2014), determined that pretreatment with mumie showed a pronounced cardioprotective effect against experimentally induced myocardial injury in rats (92). Vivek et al. (2011) they shoved that mumie had significant cardioprotective activity as it lowered the levels of serum marker enzymes (SGOT, SGPT, LDH, and CK) and lipid peroxidation and elevated the levels of GSH. Also, they demonstrated cardioprotective effects in isoproterenol-induced oxidative damage attributed to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation of membrane (93).

5.11. The Effects Profertility Reproductive and Hormonal Actions

Mumie has been used in traditional medicine as an aphrodisiac (vajikarak), geriatric tonic and by traditional healers for the treatment of male infertility (61,94). Park et al. (2006) thought that these effects might be related to

spermatogenesis and oogenesis. In their study, mumie was administered orally to 7-week-old (female and male) rats over a 6-week period. Sperm count in testes and epididymis in male rats was found to be significantly higher than control. In the female rats, the effect of mumie was estimated by the ovulation inducing activity. Study, oral administration of mumie exhibited spermatogenic and ovogenic effects in mature rats (61). An another study, thus suggest the potent androgenic nature of mumie and its role in fertility improvement against cadmium-induced infertility (94). In vitro and in vivo studies with 400 mg/mL mumie applied, provided a partial explanation for the reported effects on spermatogenesis, as well as the reports on overall fertility and libido. Moreover, it has been found to exhibit a peripheral parasympathomimetic effect in vivo. However, some scientific studies also seem to support, in part, the androgenic effect of mumie on male reproductive Health (48,95,96). Moreover, the ability of mumie to protect against radiation-induced apoptosis in rat ovaries has been reported (97).

5.12. Effects of Mumio on Osteoblastic Differentiation and Bone Regeneration

Past and recent studies have drawn attention to the effects of Mumie on osteoblastic differentiation, bone regeneration and bone fractures (64,98-100). The water-soluble fraction of mumin from Uzbekistan in Russia was evaluated for its effect on osteoblastic differentiation in cell culture experiments of human and murine mesenchymal stem. In study, have been studied the calcium deposition and expression of alkaline phosphatase, osteocalcin, core binding factor 1 (Cbfa1), and extracellular signal regulated kinase (ERK). At the end of the 14-day experiment, human bone marrow mesenchymal stem cells (hMSCs) and human fetal osteoblasts cultured with mumie (3-5 µg/mL) underwent a dramatic change in cellular morphology, which was accompanied by a significant increase in alkaline phosphatase activity, calcium deposition, and osteocalcin expression. The data showed that mumie is a potent stimulator of osteoblastic differentiation of mesenchymal stem cells and inhibitor of osteoclastogenesis (64). Azizi et al (2018) examined effect of mumie on the osteoarthritis (OA) in rat model. After 21 days, they were shown that the histopathological scores of destructive damage and synovitis decreased in the Mumie group and there was a significant difference compared to the OA group. Thus, they found that the aqueous extract of mumie reduced cartilage degenerative changes and inflammatory reactions in the synovial membrane in knee osteoarthritis (101).

5.13. Overcome High-Altitude-Related Problems

Mumie states that it is recommended for the relief of acute mountain diseases associated with high altitude such as high altitude lung and brain edema, hypoxia, insomnia, lethargy, anorexia, fatigue, stomach upset, reluctance to work, bone and muscle destruction (102,103). Mumie makes it very effective in the treatment of high altitude lung edema-like conditions and edema, it also removes excess fluid from the lungs (85). Mumie is used to treat fatigue, lethargy, and colds that are common at high altitudes. For this reason, its ability to overcome physical and mental stress was emphasized (55,85). It has also been suggested that it can be very helpful in dealing with hypoxia-like conditions in the body. Mumie has been reported to increase the oxygen carrying capacity of the blood as well as its blood purifying properties. It is stated that it helps to improve blood circulation and diffusion into tissues and maintains the required oxygen level in the body during hypoxia (36,85).

6.   Safety and Toxicity

The safety and toxicity of mumie have been evaluated in previous and recent studies in both animal and human studies. In a study in rabbits and mice, 100 mg/kg and 500 mg/kg mumie was given orally in water for 30 days. No differences were observed in the internal organs as a result of morphological or histological evaluations (98). Anisimov and Shakirzyanova (1982) administered mumie at doses of 200 mg/kg and 1000 mg/kg for 90 days in their subchronic toxicity studies in rats. After all; They reported no adverse effects on the heart, liver, kidneys, blood cells or nervous and endocrine systems. It was also found that it did not cause any embryotoxic or teratogenic Effects (104). Al-Himaidi and Umar (2003) obtained similar results in their study in mice (31). In the placebo study, 20 healthy subjects were given processed mumie (in the form of 2000 mg capsules) for 45 days. They observed that there was no significant change in heart rate, blood pressure or body weight at the end of the application. Other findings obtained from the study are that mumie does not change blood parameters such as glucose, urea, creatinine, uric acid, total protein, albumin, albumin/globulin ratio, alkaline phosphatase, ALT and AST. It was determined that mumie had no systemic toxicity under these experimental conditions (46). It has also been reported that the LD_{50} of fulvic acids isolated from mummy in rats was 1268 mg/kg when administered orally, indicating a low degree of toxicity (1). Biswas et al. (2009) in a number of human subjects studies have examined the effects of

mumie (PrimaVieW) on energy production, testosterone and spermatogenesis or spermatogenic activity, and muscle adaptation. In addition, the effects of mumie application on biochemical parameters such as urea, albumin, total protein, globulin, uric acid, bilirubin, alkaline phosphatase, ALT or AST were evaluated. They administered 35 infertile male subjects with processed mumie (100 mg in capsule form) twice daily for 90 days. At the end of the study, besides the increase in sperm count with mumie treatment, a significant increase was observed in serum testosterone (23.5%) and follicle stimulating hormone (FSH) (9.4%) levels. They observed decreases in the levels of certain biochemical parameters, such as fasting blood glucose and creatinine, in subjects treated with mumie. In addition, they found significant decreases in semen malondialdehyde levels, with significant increases in normal (18.9%) and total (61.4%) sperm count and sperm motility (12.4-17.4%) in 28 subjects. This results showed that of mumie showed antioxidant activity and under these conditions mumie does not produce evidence of systemic toxicity (48). In a pilot study where subjects were given 200 mg of processed mummy once daily for 15 days, their energy production and physical activity were examined. Treatment with Mumie was shown to significantly increase energy production and physical exercise. In addition, the increase in adenosine triphosphate (ATP), ATP/adenosine diphosphate (ADP) ratio, coenzyme Q10 (CoQ10), total adenine nucleotides, adenylate energy load and uric acid levels in whole blood confirmed energy production (34). According to Velmurugan et al. (2012) found that in 91-day administration safety experiments of mumie in rats, high-dose mumie (500, 2500 or 5000 mg/kg) did not cause any significant changes other than negligible histological changes in the liver and intestine. The weights of all organs were normal as compared to the control animals (62). In a placebo-controlled, double-blind, randomized study in dogs with arthritis, 500 mg of purified mumie was administered twice daily for 5 months. Bilirubin, AST, ALT in the liver, urea nitrogen in the kidney, creatinine and creatine kinase biomarkers of heart and muscle functions were evaluated. In addition, physical parameters such as heart rate, body temperature, respiratory rate and body weight were also investigated. The results showed no changes in physical parameters or serum biomarkers (81).

In a randomized, placebo-controlled, double-blind study involving healthy male subjects (45-55 years), the effects of purified mumie on serum testosterone levels were examined. Seventy-five subjects (37 control; 38 treated) completed the study. Subjects treated with mumie (250 mg) twice a day for 90 days had increased serum total testosterone (31.0%), free testosterone (51.1%), and

dehydroepiandrosterone (37.3%). No significant changes were observed in gonadotropic hormones (FSH and luteinizing hormone). These results supported the beneficial androgenic effects of purified mummy. (51,105,106).

7. Conclusion

In conclusion, there are some old and new animal, human and in vitro studies that make various claims regarding the therapeutic and curative efficacy of mumie. Further research based on modern scientific methods is needed on the characteristic identification properties of different mumie varieties based on their bioactive and related components to ensure its efficacy and safety. Because studies involving in vitro experiments of processed and standardized mumie have little meaning, further systematic studies, particularly in humans, are needed to elucidate the exact mechanism of action.

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OVERVIEW OF THE EFFECTS OF FLAVONOIDS ON HEALTH

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1. Introduction

Flavonoids are secondary metabolites with low molecular weight polyphenolic structure found naturally in plants. They are found in roots, stems, leaves and fruits of plants. It is known that there are substances that give the plant its color. Their most well-known properties are that they are antioxidant compounds. In addition, it has been frequently investigated in recent years due to its anticarcinogenic, antimutagenic, anti-inflammatory, etc. properties [1]. The discovery of flavonoids, which have significant effects on human health, dates to the 1930s. It was discovered as a result of studies conducted to treat scurvy disease and was called vitamin P in the early days.

In 1936, the Hungarian scientist Albert Szent-Georgye and his team treated individuals suffering from scurvy with preparations obtained from pepper and citrus peels in 1936. For this reason, although it was called vitamin P at first, the American Biochemical Association and the American Institute of Nutrition later abolished the term vitamin P in 1950 due to the fact that it did not exhibit vitamin activity [2]. Although the definition of flavonoids is widely used today, expressions such as bioactive compounds, phenolic substances are also frequently encountered in the literature. In fact, flavonoids are one of the subgroups of polyphenols that accumulate in the roots, stems, leaves and fruits of plants and act as secondary metabolites in the plant. But it is the chemical compound with the greatest diversity in the total polyphenol family [3]. In general, it is responsible for some pharmacological activities due to the fact that it is a bioactive compound, as well as physical properties such as taste, color, odor in plants. While the main sources of flavonoids are fruits and vegetables, they are also abundant in cocoa, green tea, coffee and red wine. It is also abundant in vegetables, especially colored vegetables, while spinach, broad beans, onions and olives are the most prominent in terms of flavonoid content. Plums, cherries and apples are the most flavonoid-containing plants with plenty of fruits [4].

Flavonoids, an important class of natural products, are low molecular weight metabolites. It is an aid in the treatment of many other degenerative diseases, from cancer to Alzheimer's Disease (AH) to atherosclerosis [5]. Due to this wide range of health aids, flavonoids have been the subject of frequent research in recent years. Due to the fact that they modulate enzyme activities at the cellular level, they have become an indispensable important component in nutraceutical, pharmaceutical, medical and other cosmetic fields today [1]

2. Chemical Structure and Classification of Flavonoids

Flavonoids are chemically composed of a 15-carbon phenylpropanoid chain consisting of two aromatic rings (A and B), which are connected to it by a heterocyclic pyran ring (C) as a skeleton. The structure called A, B and C is also chemically called C6-C3-C6 [6].
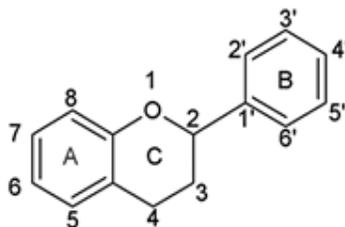
Figure 1: Chemical structure of flavonoids

The classification of flavonoids is based on the differences in this chemical skeleton. Basically, they are classified according to the degree of unsaturation and oxidation of the C ring. It is divided into 6 main groups, each of which has different properties: anthocyanidin, flavonone, flavone, flavonol, flavanol, isoflavonoid [3,4,7,8]. Each group, on the other hand, is divided into subgroups within itself.

2.1. Anthocyanidins

Anthocyanidins are the basic structures of anthocyanins. Anthocyanidins (or aglycones) consist of an aromatic ring (A) connected to an oxygen-containing heterocyclic ring (C), which in turn is connected to a third aromatic ring (B) by a carbon-carbon bond. When anthocyanidins are present in glycoside forms (attached to a piece of sugar), they are known as anthocyanins [5]. Anthocyanidins are water-soluble, non-oxidized, unsaturated flavonoids and are mainly present as pH-dependent plant pigments. Anthocyanidins are based on the basic structure of the 2-phenyl-benzopyryllium chromophore-flavyllium ion. They are hydroxylated at the C3 position in the B ring of the molecule and at the carbon atoms numbered 3, 4 and 5. The main anth Decyanidins include cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin [9]. Anthocyanins are pigments responsible for colors in plants, flowers and fruits. They are found mainly in the outer cell layers of various fruits, such as cranberries, black currants, red grapes, merlot currants, raspberries, strawberries, blueberries, blueberries, and blackberries [10]. Anthocyanins (Greek *anthos* = flowers and *kianos* = blue) are the most important pigments of veined plants; they are harmless and can be easily incorporated into the aqueous medium, which makes them interesting in terms of their use as natural water-soluble colorants. These pigments are responsible for the bright orange, pink, red, purple and blue colors in the flowers and fruits of some plants [5]. The color of anthocyanin depends on pH, as well

as on the methylation or acylation of hydroxyl groups in the A and B rings. [1]. Another important property of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cardiovascular diseases, cancer and diabetes, among others. There are several reports focusing on the effect of anthocyanins in cancer treatments, human nutrition and biological activity.[5]

2.2. Flavones

Flavones are one of the important subgroups of flavonoids. Flavones are widely available as glycosides in leaves, flowers and fruits. Celery, parsley, red pepper, chamomile, mint are among the main sources of flavones. The most important of this subclass are luteolin, apigenin, krisin and tangeritin. . While Flavones have an unsaturated C in the 2nd and 3rd C ring, there is also a ketone group in the 3-4 position [11].

2.3. Flavonone

Flavonones are another important class that are usually found in all citrus fruits, such as oranges, lemons, and grapes. Flavanones, also called dihydroflavones, have an oxidized and saturated C-ring [4]. So, unlike flavones, the double bond between Dec 2 and Dec is saturated, and this is the only structural difference between the two subgroups of flavonoids. Hesperidin and naringenin are the most important examples of this class of flavonoids. These compounds are responsible for the bitter taste of the juice and peel of citrus fruits [1]. Citrus flavonoids show interesting pharmacological effects as antioxidant, anti-inflammatory, blood lipid-lowering and cholesterol-lowering agents. In general, flavonones provide these effects with their free radical scavenging properties. Also, recent studies have shown that flavonones are the most powerful aromatase-inhibiting flavonoids [12].

2.4. Flavanol

Flavanols, commonly known as catechins, are 3-hydroxy derivatives of the flavonone group. It has a fairly wide range. The flavanol molecular structure consists of two benzene rings bonded in a linear three-carbon and oxygen heterocyclic structure (C6-C3-C6) and hydroxylated at C3. Because of this, they are also referred to as flavan-3-oller in some sources. Unlike other groups, there are no double bonds in this group at positions 2-3. This hydroxyl group can be changed by adding a gallate group, especially found in tea. Flavanols come to the fore with cis-trans isomerization and these conjugations with gallic

acid. The cis isomer at positions C2-C3 is called epicatechin, while the trans isomer is called catechin. In addition, they are called epicatechin gallate as a result of conjugation with gallic acid, epigallocatechin and Galata groups as a result of esterification epigallocatechin gallate. Like other flavonoid groups, it has important effects that help with health. Flavanols are abundant in bananas, apples, blueberries, peaches and pears [4,13].

2.5. *Flavonol*

Flavonols are involved in plant metabolism from reproductive metabolism to protective mechanisms (UV ray blocking, microbial agents, etc.) are a group of natural 2-phenyl-benzo-y-pyrane derivatives that are found almost everywhere in the plant kingdom, serving in a wide range of key roles. It is one of the most prominent flavonoid groups in this context. It is characterized by the presence of the oxide group at the C4 position and a 2,3-double bond, which allows conjugation on the C ring. It is located between the A and B rings, Dec to which it can become more sensitive to redox reactions. Flavonols and flavones are very similar, but neither oxidation of flavones to flavonols nor reduction of flavonols to flavones occurs in plants.https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/alpha-oxidation The most well-known flavonols are quarcetin, kaempferol, myristein [14].

2.6. *Isoflavonoids*

It is the group that has a more limited distribution compared to other flavonoid groups. In general, it is found in legumes such as soybeans, chickpeas. It has also been reported that it is found in considerable quantities in red clover. They contain a B-ring structurally connected to the C3 position. They can be found in the structure of aglycones and glycosides. Isoflavonoids of glycoside structure are the most well-known members of the group (daidzein and genistein). In particular, these two flavonoids are considered phytoestrogens due to the fact that they are especially similar to estrogen. It also has a high degree of antioxidant effects, like other flavonoid groups [15].

3.   Flavonoids and Their Health Effects

It has been known for a long time that the best known properties of flavonoids are antioxidant activity. In addition, in recent years, increasing interest in flavonoids has been observed to have not only antioxidant effects, but also many health-beneficial effects such as anticarcinogenic, antimutagenic, etc.

3.1. Antioxidant Effects of Flavonoids

As a result of the production of antioxidant molecules and the negative Decaying of the balance between the antioxidant defense system, the accumulation of free radicals (ROS- reactive oxygen species) increases in the body. Accumulated free radicals can lead to many more degenerative diseases, from chronic inflammation to cancer [3]. From a broad point of view, it can be seen that many effects of flavonoids are mainly related to antioxidant activity.

Flavonoids antioxidant properties are due to their basic chemical structure (flavan skeleton). Due to the phenolic hydroxyl groups present in their structure, they have the ability to directly inhibit free radicals. Antioxidant activities vary according to the presence of the -OH group in the B ring of the flavan nucleus. In general, hydrogen and free electrons here are in the structure of ROS, peroxyl, peroxynitrite, such as radicals that stabilize them and make them more stable than before [7]. In this way, they can easily chelate metal ions. They also show antioxidant activity indirectly. Flavonoid antioxidant effects are associated with activation of antioxidant enzymes (such as catalase and superoxide dismutase), suppression of pro-oxidant enzymes such as cyclo-oxygenase, lipoxygenase, NADPH-oxidase, xanthine-oxidase, phospholipase, and stimulation of the production of antioxidant enzymes and phase II detoxification enzymes. In summary, these activities help prevent many degenerative diseases, especially cancer [16]

3.2. Anticancerogenic Effects of Flavonoids

Cancer is a multi-step disease that includes physical, environmental, metabolic, chemical and genetic factors that play an important role in the formation and worsening of cancers. The mechanisms of cancer prevention of flavonoids are through a wide variety of pathways. They exert a cancer-preventing effect by inhibiting free radicals, stopping cell proliferation, inducing apoptosis and autophagy. They have also been reported to interfere with the onset and progression of cancer by modulating different enzymes and receptors in signal transduction pathways related to the reversal of multidrug resistance [4].

Cancer cells are resistant to apoptosis, a programmed cell death that is usually induced by a number of signal transduction pathways and pro-apoptotic proteins (caspases and Bcl-2 family proteins). Apoptosis is a strictly organized process, regulated by a number of signal transduction cascades and cellular proteins. Cancer and many other diseases, such as AIDS, diabetes and Parkinson's syndrome, occur as a result of imbalances in apoptotic pathways and

abnormal mechanisms. The classification of apoptotic proteins varies primarily according to their role in apoptosis. Pro-apoptotic proteins are divided into 2 classes. These are caspases and proteins of the Bcl2 family. These pro-apoptotic proteins can be down-regulated under appropriate conditions. Flavonoids can target the apoptotic signaling cascade that stimulates cell death pathways [9].

In recent years, the effects of almost every flavonoid group on cancer have been investigated. For example, it has been reported that genistein, an isoflavonoid, can regulate estrogen receptor-α expression (due to its phytoestrogen property) and change the Bax/Bcl-2 ratio by activating proliferation, differentiation, and apoptosis in MCF-7 and 3T3-L1 cells [17]. Hesperedin, a flavanone variant, is able to reduce the Bax / Bcl-2 ratio in gastric cancer cells by inducing the release of cytochrome c, the activation of caspases-3 and 9, and reducing the Bax / Bcl-2 ratio in gastric cancer cells [18]. Naringenin, another type of flavanone, has been reported to induce apoptosis through increased p53 expression, degradation of Bax and caspase-3, and survival in downregulated Bcl-2 and SGC-7901 cell lines.[19]. Flavanol catechins, especially epigallocatechin Galata, have been reported to induce apoptosis and cell cycle arrest, and to lead to cyclooxygenase-2 (COX) overexpression by inhibiting the nuclear factor kappa B (NF-kB [20]. As it can be seen, almost every flavonoid is able to show protective activity against cancer by different pathways thanks to its own chemical patterns.

3.3. Anti-Inflammatory Effects of Flavonoids

Flavonoids have anti-inflammatory properties through different mechanisms, such as inhibition of regulatory enzymes and transcription factors that have an important role in the control of various molecules involved in inflammation. It does this through various immune cells and immune mechanisms that will be mentioned in the future [21]. Chronic inflammation often precedes the development of tumors, so the anti-inflammatory effects of flavonoids can be very important in reducing inflammation and increasing the antitumor activity of immune cells. Chronic inflammation leads to tumor development by modulating the pathways of cellular transformation, survival, proliferation, invasion, metastasis and angiogenesis [22]. Flavonoids have been shown to exert an anti-inflammatory effect in such ways as immune cell regulation, suppression of chemokines, COX-2, cytokines, and pro-inflammatory transcription factors [23].

Protein kinases are involved in signal transduction during cell activation in inflammation. In this regard, some flavonoids are able to target multiple central kinases involved in multiple signaling pathways. Phosphoinositol kinase,

protein kinase C, phosphatidylinositol kinase, tyrosine inhibition of kinases such as kinase or cyclin-dependent kinase-4 by different types of flavonoids has been reported [24]. Flavonoids can inhibit phosphodiesterases such as cAMP phosphodiesterase. cAMP is a second messenger molecule that is key in the regulation of different cell functions during inflammation. High levels of cAMP have been associated with anti-inflammatory functions. Phosphodiesterase enzymes can hydrolyze CAMP to maintain normal levels. The inhibitory effects of flavonoids on phosphodiesterases are observed in the form of blocking cAMP degradation and prolonging cAMP signaling [25]. Arachidonic acid is released by the enzyme phospholipase A2 (PLA2) from phospholipids found in plasma membranes during inflammation. Arachidonic acid is then metabolized by different oxygenases such as cyclooxygenase (COX) and lipoxygenase (LOX) to produce prostaglandins, thromboxanes, leukotrienes, and other inflammatory markers [23] Flavonoids, arachidonic acid it has the potential to inhibit the enzymes involved in its metabolism by reducing the release of inflammatory mediators derived from this pathway. For example, flavonoids can inhibit the biosynthesis of prostaglandins, thromboxanes, leukotrienes through inhibition of PLA2 enzymes, COX or LOX [26]. Different properties and activities of flavonoids, cell activation, maturation, signal transduction, cytokine production or transmission pathways in several immune cells it also has a wide impact on it. For example, flavonoids, necessary for T cell activation molecules CD80, CD86 has been shown to inhibit the maturation of dendritic cells by suppressing tokens such as ripening and maturation of these cells are arranged during upward. This has a inhibitory effect on the secretion of cytokines and the proliferative response of T cells [27]. Flavonoids also have an effect on the inflammatory response of dendritic cells through the modulation of iron metabolism [28]. Some studies have reported that certain flavonoids can reduce the release of histamine or prostaglandins from mast cells, or inhibit the production of pro-inflammatory cytokines or chemokines in mast cells, neutrophils, and other immune cells [21]. It has been reported that flavonoids may have inhibitory effects on monocyte adhesion function. Among other findings also mentioned that it may Decelerate activation and proliferative response or certain immune cells involved in chronic inflammation [29].

3.4. Effects of Flavonoids on the Gastrointestinal System

The gastrointestinal tract, and especially the intestinal barrier, is very important in maintaining health. The intestinal epithelium, in addition to nutrient absorption,

provides a barrier that controls the ingress of microorganisms, their metabolic products and toxins, as well as the toxins contained in the ingested food. Due to the anti-inflammatory effect, flavonoids are able to maintain the integrity of the intestinal barrier. The microbiota contained in the intestinal lumen is very important for the whole body. Most flavonoids (with the exception of flavanols) bind naturally to sugars as β-glycosides, so they are not easily absorbed in the small intestine. Glycosylated flavonoids reach the colon, where they are actually digested. For this reason, flavonoids in the colon can affect the gut microbiome, while interacting with the bacteria present there, they can mutually modulate bioavailability and other metabolic pathways, and these processes can be beneficial for health [30].

Flavonoids and flavonoid metabolites can shape the gut microbiota by inhibiting the growth of various pathogens and increasing beneficial genera such as Bifidobacterium and Lactobacillus; they increase the conversion of primary bile acids to secondary bile acids, in turn by reducing the production of endotoxins, intestinal immunity homeostasis it can improve intestinal health by protecting and promoting the absorption of nutrients. Chronic inflammation disrupts the barrier function of the intestine and increases the exposure to endotoxin, which aggravates inflammation. Flavonoids can improve the intestinal barrier function in various ways. These;

- Inhibition of inflammatory signaling such as nuclear factor-kappa B (NK-kB) and extracellular signaling-regulated kinases (ERK)1/2,
- Downregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression,
- Reduction of oxidative stress,
- Upregulation of the intestinal hormone glucagon-like peptide (GLP)-2, which can improve intestinal barrier function,
- it is in the form of protection of the intestinal connecting barrier and its structure [31].

4.   Bioavailability of Flavonoids

Bioavailability (also referred to as bioavailability in some sources) is an important factor when investigating the function of flavonoids in human health [32]. According to the US Food and Drug Administration (FDA), the definition of bioavailability is "the speed and extent to which the active ingredient or active part is absorbed from a drug product and becomes available in its zone of action[33].

The term bioavailability is defined as the portion of digested flavonoids that is taken from regular pathways and metabolized, while today October expression of bioavailability is also defined in addition. As the definition implies, in order for a compound to be effective in metabolism, that is, to be "bioavailable", the ratio of absorption and availability in the domain is extremely important. In addition to the beneficial effects of flavonoids on health, it has been a well-known fact for many years that the bioavailability of flavonoids is low. In addition, it is a Dec fact that bioavailability can vary greatly between different flavonoid classes and compounds of a certain class. For example, it has been reported that the relative urinary excretion of anthocyanins and the uptake of daidzein are 0.3% and 43%, respectively [34].

In general, flavonoids are auxiliary metabolites that allow conjugated and hydrolyzed enzymes in the small intestine, liver and colon. It has been reported that almost all of them are conjugated to glucuronides, sulfate esters and methyl esters, and there are almost no aglycone-structured flavan skeletons in plasma. Conjugation of flavonoids occurs first in the small intestine, then they are metabolized in the liver, and the produced glucuronides and sulfate derivatives facilitate their excretion through urine and bile [33]. It is reported that 5-10% of the total intake of flavonoids, mostly those with a monomeric and dimeric structure, can be absorbed in the small intestine, usually after deconjugation reactions such as deglycosylation. The remainder reach the colon, where they are metabolized by the enzymatic action of the intestinal microbiota to compounds of different physiological significance. Flavonoids, digested in the human body, undergo phase I and phase II transformations. Phase I transformations consist of oxidation, reduction and hydrolysis. Phase II biotransformations that occur in the liver and intestines occur more intensively than in Phase I. These phase II transformations consist of conjugation reactions in which different metabolites are formed, such as methyl, glucuronic and sulfate derivatives. The fact that there are almost no aglycones is due to the fact that they are catabolized to low molecular weight compounds that can be easily absorbed [35].

The bioavailability of flavonoids depends on various factors. Its molecular weight varies depending on the degree of glycosylation, metabolic conversion rates and the degree of interaction with the colonic microflora. Each factor has a wide field of study in itself. In general, it has been observed that bioavailability decreases as molecular weight increases [33]. With some exceptions, a similar situation occurs in all classes and groups. For example, when considering classes, it has been reported that the bioavailability of anthocyanin groups is

lower compared to other groups. It is noted that the main reason for this is the rate of absorption of glycosylation and conjugation products of flavonoid classes. In general, classes in which aglycones are easily hydrolyzed can be absorbed more quickly [36]. Another bioavailability factor is the transformations in metabolism. After absorption, flavonoids interact with metabolic enzymes in enterocytes and liver. Reactions such as sulfation, glucuronidation and metilation occur. According to their basic chemical structure, the interaction with these enzymes can be separate for each class and group. Although various studies have been conducted on the bioactivity of flavonoids, these interactions have not yet been fully explained. The most well-known example in this regard is catechin. Catechin has a shorter half-life in metabolism than other flavonoids. Again, it is the most common information that epicatechin, which is in the same group, is not subjected to glucuronidation even in the colon [37]. The last factor affecting absorption is related to the range of the colonic bacterial population. Although it has been widely investigated in recent years, sufficient categorization has not yet been done. In addition, flavonoids are mostly known to reach the colon where they cannot be absorbed from the small intestine and are metabolized there. It is thought that the presence and distribution of the microbiota in the colon causes different interactions on flavonoids [33]. As a result, it is now a well-known fact how important flavonoids are for health. Despite this, concerns remain about absorption and bioavailability. In this context, studies are continuing to increase bioavailability in the light of technological developments in recent years.

5. Conclusion

The beneficial effects of flavonoids on health are now clearly known. Therefore, it is thought that flavonoids should be included more in human nutrition instead of synthetic drugs with many side effects. In order to achieve this, it is recommended to stay away from processed foods, to increase the consumption of fruits and vegetables with a balanced distribution of macro and micronutrients and where flavonoids are abundant. Although the beneficial effects were briefly reviewed based on disease and metabolism in this study, additional studies are still needed. For example, although not mentioned in our study, there is a need for more detailed investigation of these bioactive components, which have beneficial effects on health in atherosclerosis, hypertension and infective diseases. In addition, the bioavailability factor should not be ignored. Although it has beneficial effects, the bioavailability of flavonoids should also be considered. Because not all flavonoids can be completely metabolized. Today, with the help of technology,

there are studies to increase bioavailability such as encapsulation, production of combined supports with absorption enhancers, designing the chemical structure to be absorbed in the target region with bioinformatic technologies. Although the number of mentioned studies has increased, sufficient progress has not been achieved yet. Therefore, it is necessary to avoid nutritional behaviors that will adversely affect the intestinal metabolism, especially in terms of absorption, and to follow a balanced pattern in terms of nutrients.

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A BIOACTIVE COMPOUND: EUCALYPTOL

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1.   Introduction

Plants are a potential source of bioactive compounds that have always been available in the area of utilization by humans for scientific study and to the pharmaceutical and chemical industries due to their multiple administrations for their variety of activities such as antioxidant, anti-inflammatory, antibacterial, stimulative, and inhibitory features (1). One of the essential bioactive compounds, eucalyptol is also called 1,8-cineole and a naturally occurring monocyclic monoterpene ether or monoterpene ether oxide (1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane, $C_{10}H_{18}O$) and may also be synthesized by α-terpineol isomerization (Figure 1) (2). Eucalyptol was firstly described by Cloez in 1870 (3). Eucalyptol is available in large quantities in various plants, including *Artemisia herbo-alba Asso*, cardamon matou, garland flower, eucalyptus globulus, peppermint, Roma mugwort, rosemary, sage, Spanish sage, spearmint, sweet basil, and wormwood (4). Eucalyptol is certificated for man use in different fields including aromatherapy, consumption as a flavoring agent, cosmetics, and dentistry by the Food and Drug Administration (4). Eucalyptol is lipid-soluble, fast absorbed from the gastrointestinal tract and its absorption is increased in the presence of milk (2). Eucalyptol suffers oxidation and is metabolized to 2-hydroxy-eucalyptol and 3-hydroxy-eucalyptol (Figure 2), then they were excreted in the urine (2,5,6). In addition, eucalyptol may pass through the blood-brain barrier. (7,8). Eucalyptol diffuses slower by oral application or through the skin than by inhalation (9,10). Stimpfl et al. (11) showed that

prolonged inhalation exposure to eucalyptol increases cerebral blood flow, which is linked with eucalyptol levels in the blood. However, the local application of eucalyptol enhances the circulation of blood and also causes skin hyperemia (9). Recently, attention has been focused on the various medicinal and biological effects of these extracts. Eucalyptol exhibits overscaled pharmacology and biological activities including antioxidative (12), anti-inflammatory (13), antihyperglycemic (14), antihypertensive (15), antimicrobial (16), antiviral (17), antinociceptive (18), anti-tumoral (19), antipyretic (20) and analgesic (20) in a multitude of *ex-vivo* and *in-vivo* models according to reports. Thus, eucalyptol may boost the immune system, helping fight against diseases with its direct and indirect influences. The principal purpose of the current study is to cover the up-to-date literature about eucalyptol with potential influences on tissues or systems including the nervous system, cardiovascular system, gastrointestinal system, kidney, liver, pancreas, and respiratory system.

2.   The effects of eucalyptol on nervous system

The protective or therapeutic influences of bioactive compounds on the nervous system can be attributed to their talents to sympathize with nervous system signaling by way of their antioxidant, antiapoptotic, and anti-inflammatory features (21). Some researchers have suggested that eucalyptol may cross the blood-brain barrier, has a direct regulatory effect on brain enzymes and receptors, and may be utilized as a carrier to deliver medications to the brain through a microemulsion system (7,8). Eucalyptol possesses a potential depressant and antianxiety effect on the brain, in other words, it exhibits antinociceptive features (18). De Figuêiredo et al. (22) suggest eucalyptol has an essential antipsychotic-like and hypnotic-sedative influence on the brain of mice, possibly through modulation of the glutamatergic system and dopaminergic system. It has been reported that eucalyptol owns an antipsychotic activity by decreasing the symptoms of schizophrenics (22). Zheng and colleagues (23) indicated that the over-expression of P2X2 receptor mRNA and protein may be decreased by eucalyptol for rats possessing neuropathic pain in the spinal cord dorsal horn of rats. Eucalyptol was reported to attenuate subarachnoid hemorrhage-induced early brain damage in rats by alleviating apoptosis, oxidative stress, and microglial activation (8). Xu and coworkers (8) also reported that eucalyptol ameliorates subarachnoid hemorrhage-induced brain edema and neurological deficit. Ryu et al. (24) demonstrated that eucalyptol augments oxygen-glucose deprivation/reoxygenation-induced ischaemic damage by decreasing oxidative

stress in rat cortical neurons or glia. Eucalyptol has been suggested as an anti-inflammatory agent in neurodegenerative illnesses such as Alzheimer's disease (12). Khan and coworkers (12) indicated the protective effects of eucalyptol on oxidation and inflammation by reducing mitochondrial membrane potential, reactive oxygen species, nitric oxide and the concentrations of proinflammatory cytokines including interleukin (IL)-1 beta (1β), tumor necrosis factor-alpha (TNF-α), and IL-6 in amyloid β (25-35) treated cells.

3.   The effects of eucalyptol on cardiovascular system

The blood vessels and heart constitute the cardiovascular system. Endocarditis, rheumatic cardiac illness, and conduction system abnormalities lead to a large spectrum of problems that can arise within the cardiovascular system. Cardiovascular diseases include aortic atherosclerosis, cerebrovascular disease, coronary artery disease, heart artery disease, and peripheral artery disease. Unfortunately, cardiovascular disease is the most widespread leading cause of death worldwide (25). In the literature, eucalyptol is naturally found in medicinal herbs that have been emphasized to be influential against cardiovascular diseases. Soares et al. (10) showed that eucalyptol depresses myocardial contractility in a concentration-dependent way, remains the relative potentiation suggesting no interference with the sarcoplasmic reticulum function, and its depressant effect on tetanic contractions without reduction of myosin ATPase activity also suggests a reduction of Ca^{2+} influx. Thus, it was reported eucalyptol decreases contractile activity in the cardiac muscle of the rat. In addition, eucalyptol possesses an antihypertensive effect. Lahlou et al. (26) presented that the autonomic nervous system is included in the intervention of eucalyptol-induced alterations in heart rate and mean aortic pressure and also the hypotensive influences of eucalyptol could result from its vasodilatory influences directly upon vascular smooth muscle. Linghu et al. (27) had indicated that eucalyptol enhances the inflammatory phenotype of endothelial cells of human umbilical veins by mediating nuclear factor-kappa B (NF-κB) expression *ex vivo*. It has been reported that eucalyptol decreases the inflammatory infiltration and vascular cell adhesion molecular 1 expression via peroxisome proliferator-activated receptor-gamma dependent modulation of NF-κB in the sections of the thoracic aorta in lipopolysaccharide (LPS)-induced vascular endothelium dysfunction in mice (28). Moon et al. (15) indicated that eucalyptol can lower blood pressure, and that this antihypertensive influence can be related to nitric oxide regulation and oxidative stress in rats chronically exposed to nicotine. Wang et al. (29) suggested that eucalyptol

decreases the apoptosis induced by endoplasmic reticulum stress by hampering miR-206-3p, which blocks the stress-related endoplasmic reticulum protein 1 expression. Mahdavifard and Nakhjavani (30) reported that eucalyptol prevents the formation of the atheromatous lesions and enhanced kidney function in the atherosclerotic experimental model due to a decrease of glycation, inflammatory mediators and oxidative stress as risk factors for vascular complications in the atherosclerotic rats. Peng et al (14) demonstrated that carboxymethyl chitosan-coated lipid nanoparticles for eucalyptol improve the protection effects of eucalyptol on hyperglycemia-induced vascular endothelial damage via the effect of antioxidation through regulating the nuclear factor erythroid 2-related factor 2 pathway both *in vivo* and *ex vivo*. Jiang et al. (31) reported that an optimized eucalyptol-loaded self-micro emulsifying drug delivery system may alleviate LPS-induced endothelial damage and thereby, eucalyptol was suggested as a promising agent for the cure of inflammatory cardiovascular disease.

4. The effects of eucalyptol on gastrointestinal system

Natural bioactive compounds are original biological molecules of food, essentially contained in plant-based food, which exert significant beneficial influences on the man's body. Bioactive compounds have to be exposed to enzymatic hydrolysis in the gastrointestinal system or be metabolized by the intestinal microbiota to be absorbed (32). In the research on gastric secretion, eucalyptol, similar to histamine-2 receptor antagonists, has been reported as important inhibition of both total acid output as well as gastric juice volume. Santos and Rao (33) suggested that lipoxygenase inhibitory actions by alleviating the ethanol-induced gastric damage in a manner similar to nordihydroguairetic acid and the antioxidant of eucalyptol by indicating a tendency to restore the ethanol-related reduction in non-protein sulfhydryls are of prime significance in affording gastroprotection against ethanol-induced gastric mucosal injury in the rat. Moteki et al. (34) showed induction of apoptosis by eucalyptol on deoxyribonucleic acid of human leukemia cell lines (HL-60 and Molt 4B), but not in human stomach cancer cell line (KATO III). Santos and colleagues (35) investigated the effects of eucalyptol in rats with acute colitis. They discovered that eucalyptol reduced myeloperoxidase and restored glutathione (GSH) in the colon, suggesting its potential as an anti-inflammatory and ulcer-prevention agent. Murata et al. (19) indicated that eucalyptol suppresses colorectal cancer proliferation by activating p38 and inactivating survivin and Akt, in other words inducing apoptosis, in human colon cancer cell lines RKO and HCT116. They

suggested that eucalyptol's anticancer efficacy stems from its ability to induce apoptosis in human colorectal cancer cells both *ex vivo* and *in vivo*. Caldas et al. (36) indicated the role of eucalyptol as an essential ulcer healing agent and demonstrate the involvement of antioxidant and cytoprotective mechanisms in the gastroprotective influence of the compound. Moreover, Caldas et al. (36) reported that eucalyptol augments mucus, prevents biochemically non-protein sulfhydryl groups depletion and decreases myeloperoxidase and lipid peroxidation, reduces histologically in the lesion of the gastric area, promotes important regeneration and restoration mucus in glandular cells and promotes augment in cell proliferation in the gastric mucosa in chronic ulcer model. It has been reported that eucalyptol microcapsules possess protective effects against gut microbiota imbalance and inflammation related to weight loss induced by heat stress in Broiler chickens and it was suggested that eucalyptol microcapsules can be a good feed supplement to guard against heat stress damage (37).

5.   The effects of eucalyptol on kidney

Eucalyptol, a natural bioactive chemical, could be a potential adjuvant therapy for the management of pathological disorders such as acute or chronic kidney disease, which is one of the non-communicable degenerative illnesses that is a global public health issue (38). Kim et al. (39) reported that eucalyptol inhibited Snail1 and -catenin, preventing tubulointerstitial fibrosis and tubular epithelial derangement in diabetic models of kidney tubular cells, and it blocked glucose-induced loss of E-cadherin, expression of N-cadherin and -smooth muscle actin, and induction of connective tissue growth factor and collagen IV. Kim and Kang (40) indicated that eucalyptol enhanced focal adhesion formation and actin cytoskeleton integrity in diabetic kidneys. Kim and colleagues (41) reported that eucalyptol reduces the induction of Rho GTPases of Rac1, ROCK, Cdc42, and Rho A in glucose-loaded podocytes and in diabetic mouse kidneys. Thus, in the light of these studies, it was suggested that eucalyptol can be a potent kidney protective agent counteracting diabetes-related podocyte detachment and disruption of focal adhesion proteins and also therapeutically counteract the dysfunction of barrier and proteinuria.

6.   The effects of eucalyptol on liver and pancreas

Experimental research found that a great number of bioactive compounds, including anthocyanin, curcumin, resveratrol, and tea polyphenols, could ameliorate hepatic steatosis, apoptosis, oxidative stress, and inflammation

in liver diseases (42). It was indicated that eucalyptol protects mice against the D-galactosamine/LPS-induced shock model of liver damage through the reduction of TNF- α production and suggested that it can be a promising agent to fight septic-shock-related pathologies (43). Cho (44) demonstrated that eucalyptol reveals lipid-lowering and antioxidant effects due to strengthening the high-density lipoprotein function thus augmenting reverse cholesterol transport with respect to anti-inflammatory and anti-atherosclerotic activities in the hypercholesterolemic zebrafish model. Murata et al. (45) revealed that eucalyptol ameliorates non-alcoholic steatohepatitis by downregulating collagen 1a1 expression and improving liver fibrosis in tensin homolog-knockout mice. Al-Musawi et al. (46) demonstrated that eucalyptol owns antioxidant effects by decreasing thiobarbituric acid reactive substance and increasing GSH level and catalase activity against long term dioxins like compound polychlorinated biphenyls toxicity in the liver of chicken.

In a study, that investigated a meta-analysis and systematic review on the global incidence of acute pancreatitis by Iannuzzi and coworkers (47), it was reported that acute pancreatitis is a widespread disorder with importance related to morbidity and mortality in the world. In addition, they also suggested the increase in acute pancreatitis can be correlated with rises in obesity and metabolic syndrome, which are related to gallstone illness (47). Thus the diet can play an important role in the protection from acute pancreatitis. Lima and colleagues (48) indicated that eucalyptol reported enhances cerulein-induced acute pancreatitis in an experimental mouse model by oral application of eucalyptol due to pro-and anti-inflammatory cytokines modulation, oxidative stress, and the activity of NF-κB.

7.   The effects of eucalyptol on respiratory system

Nowadays, the diseases of the respiratory system, particularly the pandemic of respiratory system infectious illnesses and chronic lung diseases have still continued the studies related to them because of their high morbidity and mortality rates and their poor prognosis. In the literature, it has been found pretty much studies about protective and therapeutic influences and molecular mechanisms of bioactive compounds against the illnesses of the respiratory system (49). Eucalyptol influences cilia activity in the epithelium of the respiratory, enhances the secretions transport, aids to clear the tract of the respiratory, and eases expectoration. Wittmann and coworkers (50) reported on bronchodilators' effects in asthma patients following oral administration with

eucalyptol. It was presented that in a work, in sicks with mild and moderate asthma, additional treatment with eucalyptol also ameliorates the function of the lung and suppresses in *vitro*-stimulated the production of inflammatory mediators in short-term cultures of peripheral monocytes (51). Juergens et al. (52) showed an anti-inflammatory activity of eucalyptol in steroid-dependent bronchial asthma in clinical trials. Previous studies (52,53) reported evidence for the involvement of eucalyptol in the management of airway mucus hypersecretion through inflammatory cytokine suppression, suggesting that long-term eucalyptol administration may reduce asthma, sinusitis, and chronic obstructive pulmonary disease exacerbations. In addition, concomitant treatment with eucalyptol was demonstrated to decrease exacerbations as well as dyspnea and ameliorate lung function in a placebo-controlled double-blind trial with 242 chronic obstructive pulmonary disease patients (54). Bastos and colleagues reported that inhaled eucalyptol decreases inflammatory parameters such as IL-1β and TNF-α concentrations in the airways of ovalbumin-challenged Guinea pigs (55). The primary ingredient in the clinically approved medicine Soledum®, eucalyptol, is well-established for treating airway disorders such as chronic sinusitis and bronchitis, chronic obstructive pulmonary disease, and bronchial asthma (56). Zhao and coworkers found that eucalyptol reduced IL-1, TNF-, toll-like receptor 4 and NF-B p65, myeloperoxidase, the number of inflammatory cells, neutrophils, and macrophages, in bronchoalveolar lavage fluid (BALF), as well as the protein content in BALF and the lung wet/dry weight ratio, and increased IL-10 in lung tissues after acute lung injury induced by LPS (13). Eucalyptol has been determined to protect against influenza A virus infection in mice by reducing IL-4, IL-5, IL-10, and monocyte chemoattractant protein-1 in nasal lavage fluids and IL-1β, IL-6, TNF-α, and interferon-gamma in the lung (17). Furthermore, Li and colleagues (57) suggested that eucalyptol provides cross-protection against the influenza virus when given in combination with inactivated influenza viral antigen in a mouse model. It was shown that intervention of eucalyptol mitigates the ongoing inflammatory process in the airways and enhances the cigarette smoke-induced lung damage by suppressing intercellular adhesion molecule-1 gene expression in the diseased lungs (58). Yu and colleagues (59) demonstrated that eucalyptol treatment supports the elimination of bacterial organisms from cigarette-exposed lungs by reducing ciliated cell damage and inhibiting mucin 5AC expression in the lungs. Kennedy-Feitosa et al. (60) showed that eucalyptol induced lung repair by restoring redox markers concentrations and extracellular matrix components to normal

concentrations and it promoted lung repair in mice following emphysema by induced cigarette smoke.

8.   Conclusion

Eucalyptol is a bioactive compound and possesses remarkable properties. Due to its biological and pharmacological activities, such as anti-apoptotic, anti-inflammatory, anti-oxidative, antihyperglycemic, antihypertensive, antimicrobial, antiviral, antitumoral, antipyretic, and analgesic, eucalyptol can be used as a component of many drugs used to treat a variety of diseases. It may be also considered the use of therapies supported by eucalyptol in the early stages of the diseases. Overall, it can be concluded that eucalyptol can be a natural alternative for boosting the immune system and defeating diseases. Thus, further research is required clearly elucidate the mechanisms of its known properties on each tissue of the body. Moreover, several issues have to be further researched especially the reliance and effective doses of eucalyptol.

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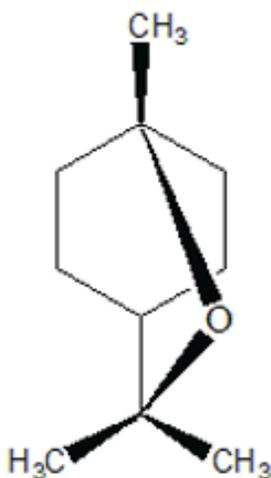
Figure 1. Chemical structure of eucalyptol
(1, 3, 3-Trimethyl-2-oxabicyclo [2, 2, 2]octane) (1).
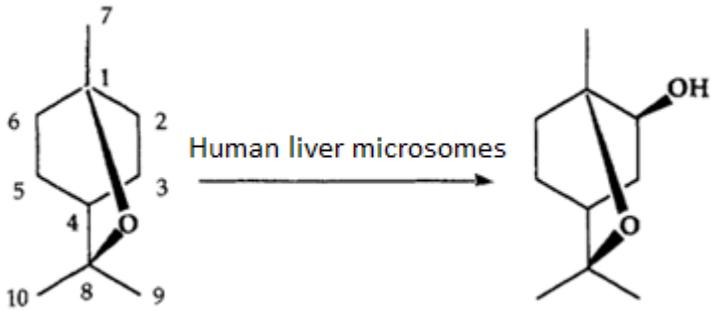
Figure 2. Eucalyptol biotransformation by human liver microsomes. 1: Oxidation of eucalyptol and 2: 2-exo-hydroxy-eucalyptol (5).

CURCUMIN: A HEALTHY SPICE FROM ASIA

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1.   Introduction

1.1. History

Curcumin (CUR), a spice widely used in Asian cuisine, is isolated from the root stem and rhizome of *Curcuma longa* (turmeric) (1-9). Turmeric, a perennial herbaceous plant belonging to the *Zingiberaceae* family with 133 different species worldwide, is endemic to South Asia, Indonesia, and India (8,10,11). It grows spontaneously in South Asia and Africa in tropical climates with high rainfall and temperatures between 20 and 35°C. (8). Turmeric has been used in traditional medicine since ancient times in Asia and is widely used today in foods, cosmetics, and the drug industry (2). For the past 4000 years in India, turmeric has been used under the name "Indian turmeric" or "Golden Spice" because of its bright yellow color. The root of this plant is the most valuable part for medicinal applications, especially in South Asia, and is a popular Asian spice used especially in Thailand, Pakistan, and India (12).

1.2. Discovery and Chemical Structure

CUR was first isolated in 1815 in the lab at Harvard College by two scientists, Pelletier and Vogel, after which research interest in CUR increased. With a molecular weight of 368.38 g/mol, CUR's chemical formula is $C_{21}H_{20}O_6$ (1,3,6,11). The International Union of Pure and Applied Chemistry has identified CUR, also known as diferuloylmethane (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, as a biologically active natural polyphenol obtained from the rhizome of *C. longa* (1,3-6,8,10,11,13-16).
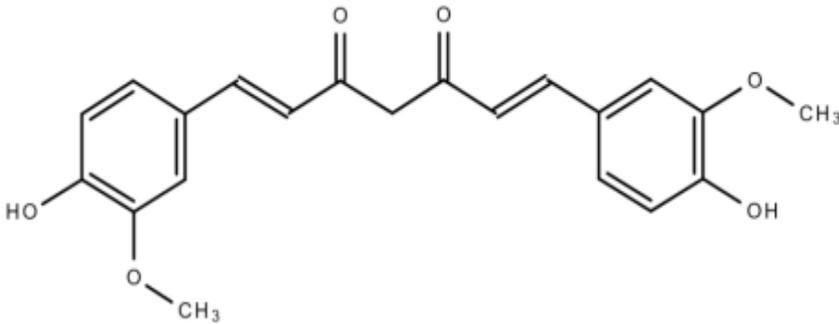
Figure 1: Chemical structure of curcumin.

It has been reported that CUR was obtained as a pure compound in 1842 (11,12). Its chemical synthesis began with Lampe (11); its chemical structure was described in 1910 and it was synthesized in 1913. In 1949, CUR's antibacterial characteristic was reported (8,12,18). More detailed research was initiated after German scientists determined the therapeutic potential of the oils extracted from the roots of the turmeric plant (11).

Turmeric contains essential oils, such as zingiberene, and coloring agents known as curcuminoids (2). Depending on its biological origin, soil fertility and place of cultivation, turmeric contains between 1.5 and 9% CUR (2,11,12). In addition to CUR, turmeric contains two other curcuminoids-dethoxycurcumin and bisdemethoxycurcumin-in smaller amounts (5,16). Curcuminoids make up 2-4% of dried turmeric root powder. CUR can be applied as turmeric, turmeric concentrates, pure (95%) curcuminoids, or CUR alone (5) and is responsible for turmeric's pure yellow color (8,12). Commercial CUR products contain a mixture of curcuminoids, including ~77% CUR, 17% dethoxycurcumin, and 3% bisdemethoxycurcumin (2,3,8,11,13,16), and it has attracted great interest in recent years because of its positive pharmacological activities. Because of the conjugated double bonds in its chemical structure, CUR acts as an effective

electron donor to counteract the production of reactive oxygen species in many redox reactions (4).

1.3. Mechanism

Turmeric powder has been widely used since ancient times in domestic medicine to treat various diseases, such as cough, diabetic ulcers, liver diseases, biliary tract disorders, rheumatism, sinusitis, and anorexia (12). One of its ingredients, CUR, exhibits a wide range of beneficial effects as an antioxidant, anti-inflammatory, antiproliferative, antitumor, anticancer, antibacterial, antimicrobial, antifungal, antiviral, antineoplastic, antidepressant, and analgesic compound; protects tissues and regulates metabolism; and has chemoprotective, cardioprotective, and hepatoprotective properties (3-7,9,11-13,16,17,19,20). In addition, the consumption of CUR has beneficial/therapeutic effects for various human diseases, such as metabolic syndrome, skin diseases, cancer, intestinal inflammation, arthritis, fatty liver disease, cardiovascular diseases, neurodegenerative disorders, obesity, diabetes, and premenstrual syndrome (1,4,6,7,9,10), and it has a long history of use in Asian countries against skin conditions, including acne and psoriasis (3), all resulting from CUR's antioxidant and anti-inflammatory effects (1,4,15). It has been suggested that CUR has a beneficial effect on disorders of the nervous system, cardiovascular system, digestive system, respiratory system, endocrine system, and renal system (10).

1.4. Solubility

CUR is a fat-soluble compound; therefore, it is nearly insoluble in water at room temperature, or in acidic and pH neutral environments (5.6). CUR is easily soluble in organic solvents, such as acetone, acetic acid, dichloromethane, ethyl acetate, dimethylsulfoxide, chloroform, methanol, and ethanol (2-4,12). Its poor solubility in acidic or neutral environments and its instability under alkaline conditions limits CUR's use (19). In addition, clinical use of CUR is limited by its low water solubility, which results in poor absorption following oral administration (2,3,8,15,17). CUR exhibits keto-enol tautomerism depending on the pH of the solution. The keto form is essential at pH < 7, and the enol form at pH > 7 (14,16).

1.5. Bioavailability

Various CUR formulation strategies have been proposed that increase its aqueous solubility and oral bioavailability and provide greater therapeutic

efficacy (1,2,9,15,17,19). These include combining CUR with hyaluronic acid, polyethylene glycol, sodium alginate, polyurethane, or various polymers encapsulated in the hydrophobic core of different micelles and liposomes (9). Different delivery systems, such as micelles, liposomes, phospholipid complexes, microemulsions, nanostructured lipid carriers, and biopolymer nanoparticles, have been developed to increase CUR's bioavailability (4). Other strategies that have been developed to increase CUR's bioavailability are based on combining CUR with other molecules. For example, administering CUR together with black pepper and piperine, an alkaloid of black pepper, have been suggested (2.15). CUR's low solubility and its ability to be rapidly metabolized together with some other pharmacological problems can also affect its absorption by the body's digestive system. For example, one study (21) has found that the absorption of CUR in the gastrointestinal tract of rats was approximately 25%. It has been reported that CUR's oral bioavailability is approximately 1% in rats (8,19). It was also found that CUR can be determined in plasma 15 min after intraperitoneal administration and detected in the liver, spleen, intestines, and kidneys after 45 min; however, although CUR effectively crosses the blood–brain barrier, only traces are seen in the brain (8).

1.6. Metabolism

No significant toxicity has been reported from oral administration of CUR; however, some gastrointestinal disorders can occur, with high concentrations detected (4,10). High oral doses of CUR are poorly absorbed from the gastrointestinal tract and rapidly reach high levels in the blood within 1 h after administration (2). CUR is rapidly metabolized; therefore, this natural compound has low bioavailability (5,6,7,9). In fact, it is metabolized in the liver (glucuronidation) and to a lesser extent, in the intestine. Glucuronidation dominates this metabolic pathway (8,19). CUR is rapidly metabolized by the liver and excreted with feces (3,8), after which it is excreted in the urine as it conjugates with sulfate and glucuronides in the liver (8). In addition, CUR is metabolized by various enzymes in the intestines to produce several metabolites. Although dihydrocurcumin, tetrahydroxycurcumin, hexahydrocurcumin, and octahydrocurcumin are produced during phase I metabolism, sulfate and glucuronide O-conjugated compounds are produced in phase II metabolism (7,16).

2.   CUR's Role in Health

The use of natural products to treat autoimmune and inflammatory diseases has been increasing. Although these products show important anti-inflammatory effects, they also offer a wide variety of bioactive compounds, one of which is CUR. Several types of turmeric have been used to treat various diseases since ancient times (20). CUR, a compound found in turmeric, can be oxidized by free radicals and oxyradicals (5). Because of its hydrophobicity, CUR tends to penetrate the cell membrane and bind to the fatty acyl chains of membrane lipids through hydrogen bonding and hydrophobic interactions, which reduces CUR's availability in the cytoplasm; therefore, its uptake at the cellular level is low (17).

Oxidative stress is associated with many chronic diseases (15), and because CUR can interact with a variety of molecular targets involved in chronic diseases, it has broad implications for medical application (19). CUR scavenges free radicals and inhibits malondialdehyde (MDA) production to improve antioxidant levels in sick individuals sensitive to oxidative stress; however, reducing this oxidative stress depends on the dose and duration of treatment (4).

3.   CUR's Role in Neurological Diseases

Alzheimer's disease is a chronic neurodegenerative disease characterized by the presence of hyperphosphorylated tau protein in neurofibrillary tangles, extracellular accumulation of amyloid beta peptides in the hippocampus, selective neuronal loss, loss of memory, and cognitive impairment. Parkinson's disease on the other hand, is a type of movement disorder associated with a lack of dopamine, a neurotransmitter (10). CUR's anti-aggregation properties, it has been known to reduce Alzheimer's pathology (10,22). It has been reported that CUR administration in rotenone-induced Parkinson's disease in mice has increased the levels of superoxide dismutase, catalase, and glutathione in the brain homogenate and reduced MDA and nitrite (10).
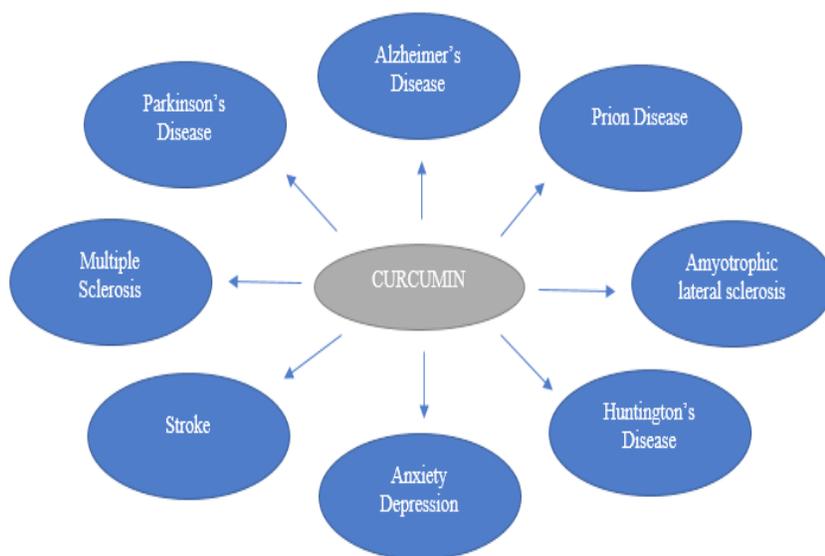
Figure 2: Curcumin in neurological diseases.

It has been determined that CUR has a therapeutic effect in many neurological diseases (Fig. 2). CUR is a pleiotropic molecule that not only directly binds and limits the aggregation of misfolded proteins in neurodegenerative disease but also maintains homeostasis of the inflammatory system, cleans toxic clumps from the brain, scavenges free radicals, and chelates and induces iron (11).

4. CUR's Role in Cancer

Phytochemicals are plant-derived chemicals with various physiological activities that are suggested to be effective in cancer treatment. CUR, a phytochemical isolated from turmeric, is widely used in traditional medicine and has been determined to be a safe food additive by the U.S. Food and Drug Administration. Extensive clinical trials have been conducted using CUR mainly as an adjuvant for cancer treatment, and positive results have been suggested following its use (23,24). This phytochemical is a highly effective candidate in the treatment of cancer, either as a single drug therapy or in combination with other therapeutic agents. This natural compound also has the capacity to affect a variety of molecular targets and signaling mechanisms associated with various types of cancers (6), providing preventive and therapeutic effects on these cancers (25). Combining CUR with natural compounds, such as resveratrol, lycopene,

gingerol, and folate, has gained a lot of attention as an alternative to traditional treatments. These natural compounds have been shown to have chemopreventive and/or anticancer activities with minimal side effects (2,3).
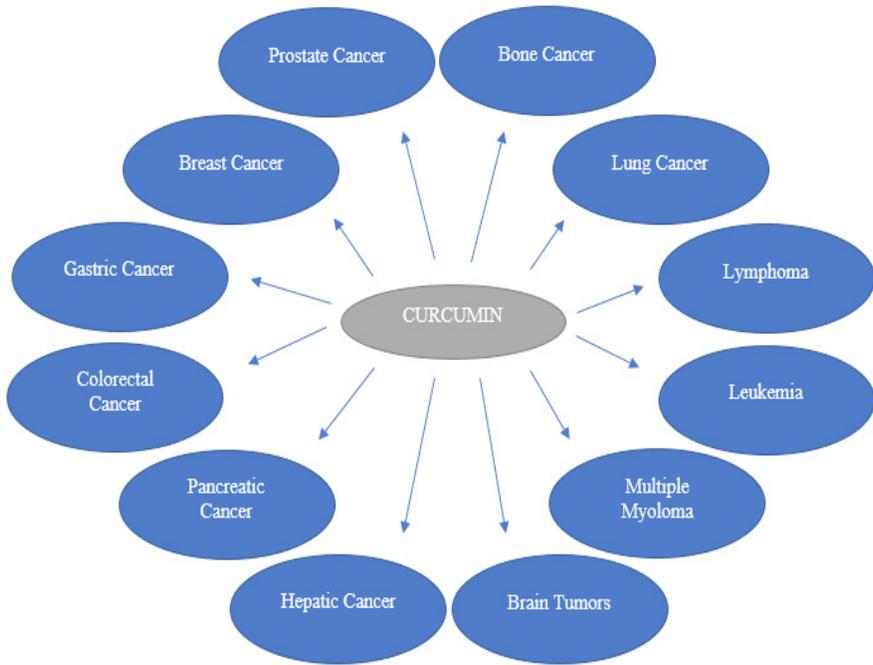
Figure 3: Curcumin use in treating some cancers.

The main mechanisms of action by which CUR exhibits its anticancer activity includes inhibiting the proliferation and invasion of tumors by inducing apoptosis and suppressing various cellular signaling pathways (17). It has strong antioxidant activity at low concentrations; however, at higher concentrations, CUR can act as a prooxidant compound favoring cancer therapy (7). It has been reported that CUR may be effective at all stages of cancer, such as initiation, progression, and metastasis, and that it can control the disease through various mechanisms (19). As with other dietary polyphenols, CUR prevents the effects of toxic damage to different tissues and interferes with key signaling pathways associated with cancer by directly targeting proteins or regulating gene expression (4). Plant-derived-natural compounds are attracting interest in cancer treatment because of the emergence of drug resistance, and because cancer continues to threaten the lives of people throughout the world, attempts are ongoing in its treatment (7).

Although CUR is believed to be effective in cancer treatment, its clinical use is limited because of its low bioavailability and low water solubility (1). It may also interfere with systemic iron metabolism, which indicates some limits to its application in patients with chronic diseases or anemia (4).

5.   Conclusion

Various studies have proved the beneficial effects of CUR against different diseases. It is gaining worldwide attention mainly because of its multiple health benefits that appear to act through its antioxidant and anti-inflammatory mechanisms; however, to increase the effectiveness of these benefits, CUR's bioavailability must be increased. Low doses of CUR used regularly may also provide health benefits for people who have not had any diagnosed health problems. To better elucidate CUR's actual biological activity and increase its bioavailability, we suggest that more studies on this subject be conducted.

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UTILIZATION *OF NIGELLA SATIVA* (BLACK SEED) IN LIVESTOCK PRODUCTION

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1. Introduction

Nigella sativa (Black seed), which is in the group of medicinal plants, has a highly well-known history. The reason why black seed is so important lies in the strenuous compounds of its seeds. *N. sativa* seeds have been used in ancient Egypt and Greek civilizations as a therapeutic against various diseases (head and toothache, diuretic, increasing breast milk, carminative, anti-constipation, antipyretic, expectorant) (1, 2). *N. sativa* has a unique appearance. It is 50-60 cm high and having white flowers. *N. sativa* has a self-reproducing fruit capsule consisting of numerous white seeds. After ripening, the fruit capsules open and the seeds inside become black by exposure to air (Figure 1).

Figure 1. *Nigella sativa*'s plant, seeds and oil.

Its seeds are triangular shape, black in color and contain abundant oil (3). The seeds, which have numerous medicinal uses, have been subjected to various chemical and medical experiments. The seeds consist of 28-36% crude oil, protein, saponins, various alkaloids, and essential oils (0.4-2.5%). Within the unsaturated fatty acid profile; arachidonic, eicosadienoic, linoleic and linolenic acids are present (4, 5). Saturated fatty acids are stearic, palmitic, and myristic (Hajhashemi et al., 2004). The active components of the essential oils contained in the seed contain thymol, dithymoquinone, thymoquinone and thymohydroquinone (6, 7). There are 4 alkaloids reported as components of its seeds. These are nigellisin, nigellidin, nigellimine, and nigellimine's N-oxide (8). *N. sativa* also contains a large number of amino acids; glutamic acid 22%, aspartic acid 10%, arginine 9%, leucine 7%, glycine 7%, pyrroline 6%, valine 5%, alanine 4%, phenylalanine 4%, isoleucine 4%, threonine 4%, lysine 4%; serine 4%, tyrosine 3%, histidine 3%; methionine 1.5%, cystine 1.2%, and tryptophan 0.8% (9, 10). Apart from these, it has been reported that α-heredinin in the structure of the seed has antitumor activity (11).

Previous studies have shown many medicinal properties of *N. sativa* seeds, such as immunomodulatory, anti-inflammatory, anti-microbial and anti-oxidative effects (Figure 2; 12). It is known that components, such as thymoquinone, anethole, carvacrol, and 4-terpineol own very important antioxidant properties (13). According to Salomi et al. (14), *N. sativa* seeds had minimal cytotoxicity against normal lymphocytes but exhibited a potent cytotoxic effect on carcinoma, lymphoma and sarcoma. In another study, its seeds were seen to inactivate mammary tumor cells in vitro (15).
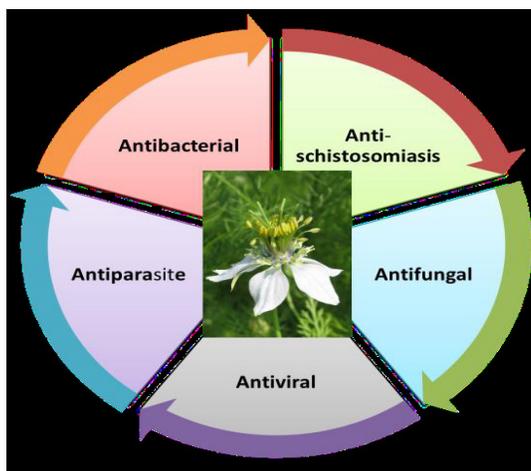
Figure 2. Potential medicinal effects of *Nigella sativa* (16)

Black seed has been reported to possess an inhibitory effect for several pathogens. These are *Staphylococcus aureus, Trueperella pyogenes, Corynebacterium pseudotuberculosis, E. coli, Listeria monocytogenes, Yersinia enterocolitica, Brucella abortus, Corynebacterium renale, Pasteurella multocida and Mannheimia haemolytica* (17). A study (18) reported that essential oils in *N. sativa* seeds are effective against gram negative (*Pseudomonas aeruginosa, E. coli*) and gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*). In a study, oils of black seed have potent antimicrobial role against L monocytogenes greater than gentamicin (19). In another study, methicillin-resistant *Staph. aureus* (MRSA) strains have also shown to have a highly inhibitory effect against *N. sativa* seeds (20). In addition, *N. sativa* oils were resulted to have a perfect antifungal potential against Aspergillus spp. and oil applications significantly reduced viral accumulation in the liver (21). Researchers (22) also tested the effects of black seed oil on the proliferation and AFB1 output of *A. flavus* and *A. parasiticus* fungi. In the aforementioned study, it was found that *N. sativa* oil application inhibited AFB1 formation by 50-58% and 32-48%, respectively. In different human and animal studies, black seeds have also been showed to significantly reduce serum cholesterol and lipoprotein levels (23, 24, 25, 26).

2.   Nigella sativa (Black seed) Utilization in Ruminants

A study was conducted to examine the influences of black seed meal (32.8% CP, 5.34 Mcal/kg ME) feeding on growth performance of lambs (27). Diets containing 150 g of black seed meal/kg DM increased the intakes of DM, CP, NDF and ADF (P<0.05). In addition, BW, ADG, and feed efficiency was found to be greater (P<0.01) in black seed meal containing diets. Authors suggested that BSM can be used as protein and energy source in lamb rations. The study's data is summarized in Table 1.

Table 1. Influences of black seed meal (BSM) feeding on nutrient intakes, digestibilities and growth performance of lambs

Item	Control	**BSM**	p-value
Intake, g/d			
DM	1034	**1124**	0.04
CP	167	**183**	0.05
NDF	302	**347**	0.01
ADF	201	**223**	0.04
Ether extract	19	**39**	0.001
Digestibility, %			
DM	75.2	**78.9**	0.02
CP	73.6	**78.1**	0.04
NDF	58.6	**56.8**	0.13
ADF	53.5	**52.8**	0.82
Ether extract	74.9	**85.6**	0.002
Growth			
Final weight, kg	31.7	**36.3**	0.002
Average daily gain, g/d	217	**272**	0.001
Feed efficiency	4.75	**4.17**	0.01

A research was also performed the influences of feeding *N. sativa* meal (NSM) on wool yield and quality of male lambs (n= 21) from 90 to 300 days of age (28). Lambs were fed with NSM either at 5 or 10% of diet DM. The results showed that feeding NSM increased wool yield, body weight gain and fleece weight. In addition, wool quality parameters (staple elongation rate, staple strength, staple length and point of staple break) were also improved significantly ($P<0.01$) with NSM feeding. The data are shown in Table 2.

Table 2. Wool quality parameters of lambs fed with two levels of *Nigella sativa* meal (NSM)

Wool quality parameter	Control	**5% NSM**	**10% NSM**
Greasy fleece weight, kg	2.18[b]	**2.52[a]**	**2.67[a]**
Clean yield, %	51.4[b]	**54.5[a]**	**55.2[a]**
Fiber diameter, μm	33.2[c]	**34.9[b]**	**36.7[a]**
Medullation index	19.8[b]	**21.9[a]**	**21.9[a]**
Prickle factor, %	41.3[b]	**47.9[a]**	**48.1[a]**
Crimps/cm	2.96[a]	**1.84[b]**	**1.53[b]**
Staple length, cm	5.55[b]	**6.45[a]**	**6.68[a]**
Staple strength, N/ktex	43.9[b]	**51.7[a]**	**50.7[a]**
Staple elongation rate, %	39.1[b]	**45.6[a]**	**46.2[a]**

Different superscripts in the same row differ significantly

In another *Nigella sativa* (NS) research, 23 small ruminant study databases were evaluated in a Meta-Analysis (29). Results showed that interaction between NS levels and small ruminant species were tended to be significant and increased average daily gain linearly as dietary NS levels increased ($p<0.01$, $R^2=0.54$). In addition, dry matter intake in lambs was higher as dietary NS levels increased ($p<0.01$, $R^2=0.96$). Nitrogen intake and digestion were also found to be significantly increased ($p<0.01$) as NS inclusion rate was increased. Authors also resulted that there was a strong positive relationship and increments between NS supplementation and IgA ($R^2=0.92$) and IgG ($R^2=0.94$) ($p<0.05$) concentrations.

The antibacterial effects of black seed extract against cow's mastitis pathogens were also examined *in vivo* and *in vitro* (30). Clinically mastitis cows were administered with black seed extract intra mammary. For the in vitro study, the extract was used against for those isolated bacteria. Both experiment confirmed that black seed extract had a substantial inhibitory result. The data of the experiment are presented in Table 3 and 4, respectively.

Table 3. Antibacterial effects of *Nigella sativa* seed
extract against cow's mastitis agents in vivo (CFU in 1 ml of milk × 1000)

Bacteria	No. of case	*N. sativa* concentration, %	Before treatment	After treatment	p-value
S. aureus	10	3	51.0	17.3	<0.05
β-hemolytic streptococci	11	3	38.2	18.7	<0.05
*Enterococci (*Group D *streptococci)*	15	6	45.9	10.7	<0.01
E. coli	12	6	19.2	0	<0.05
S. aureus	5	6	42.0	0	<0.01

Table 4. Antibacterial effects of *Nigella sativa* seed extract
against cow's mastitis agents in vitro (Agar dilution method)

N. sativa concentration (mg/ml)	*Enterococci (*Group D *streptococci)* Growth %	Growth inhibition %	*E. coli* Growth %	Growth inhibition %	*S. aureus* Growth %	Growth inhibition %
0	100	0	100	0	100	0
20	25	75	90	10	30	70
40	0	100	30	70	0	100
80	0	100	0	100	0	100
160	0	100	0	100	0	100

3. Nigella sativa (Black seed) Utilization in Poultry

Potential effects of black seed (*Nigella sativa*; NS) in 3 levels (1, 2 and 3% of diet DM) were also studied in layers diet (31). The tested variables were feed efficiency, egg production, and shell grade. They found no effects on egg yield, body weight, feed efficiency, and feed intake, albumen and yolk indexes at any level of black seed. However, diets offered with 3% *N. sativa* effected the egg's albumen weights (p<0.05). In addition, dietary black seed supplementation at 3% enhanced egg weights. The data are presented in Table.

Table 5. The effects of black seed (*Nigella sativa*)
supplementation on laying hens performance

Item	Dietary Treatments			
	Control	1% NS	2% NS	3% NS
Feed intake, g/d	132.1	132.2	132.8	132.6
Egg weight, g	60.5[b]	60.9[b]	62.6[ab]	63.6[a]
Albumen weight, g/egg	37.6[b]	37.8[b]	39.9[ab]	40.5[a]
Percentage of eggs %				
Albumen	62.1[b]	62.0[b]	63.7[ab]	63.8[a]
Shell	12.3[a]	12.1[ab]	11.6[ab]	11.5[b]

In a study on 49-day-old broilers fed with broken or whole *N. sativa* (32), data of blood parameters, growth abilities, and carcass variables were tested. Ration groups were control, 1.5, 2.0, 2.5, and 3.0% broken or whole *N. sativa* (NS) respectively. Higher body weight gain and feed conversion ratio were detected in chicks fed with 1.5% broken NS (P<0.05). In chicks fed with diets containing 3.0% broken or whole NS, plasma cholesterol and triglyceride levels decreased while plasma HDL levels increased (P<0.05).

Similarly, a study was performed to compare the effect of various levels of NS addition (5, 10 and 20 g/kg) in broiler diets for immune response and blood biochemical parameters (33). Results indicated that dietary supplementation of NS at 10 and 20 g/kg improved the broilers plasma lipid profile and antibody-mediated immunity. The data are presented in Table 6.

Table 6. The effects of dietary *Nigella sativa* addition on plasma biochemical parameters and antibody titers of in broiler chickens at 42 d

Item	Experimental *Nigella sativa* treatments, g/kg				
	Control	5	10	20	P<
GLU, mg/dL	245	239	238	247	NS
TG, mg/dL	69.2[a]	59.5[ab]	60.4[ab]	54.3[b]	0.05
TC, mg/dL	146.0[a]	141.0[ab]	126.5[b]	121.0[b]	0.01
HDL-C, mg/dL	58.7	60.8	59.1	61.2	NS
LDL-C, mg/dL	69.4[a]	62.3[ab]	57.2[ab]	50.4[b]	0.01
Initial IG titer	4.57[d]	5.08[c]	5.21[b]	6.10[a]	0.01
Second line IG titer	6.32[b]	6.56[b]	7.42[a]	7.92[a]	0.01

4. Conclusion

N. sativa seeds are frequently preferred in poultry due to their rich unsaturated fatty acid profiles (linoleic, oleic, eicodadienoic and dihomolinoleic). Positively affected parameters are; egg quality (improved egg production, egg weight and shell quality); low cholesterol level in egg yolk; antibacterial (reduces the presence of E. coli) and antiviral (inhibits Infectious Bronchitis virus) properties. For clinical use in veterinary medicine, it may be advisable to include the seed and its active ingredients in modern pharmaceutical products. In addition, the ban on the use of antibiotics and hormones in the laying hen and broiler industry has made using *N. sativa* seeds an alternative. Therefore, it is understood from the literature that the *N. sativa* seed has a great multi-faceted (pharmacological, physiological and biochemical) potential.

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A BIOACTIVE MICRONUTRIENT; RESVERATROL

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1.   Introduction

Plant-derived chemicals called phytoestrogens have recently gained great importance with epidemiological studies. Phytoestrogens occur naturally and contain phenolic groups. These phytochemicals can be divided into four main groups: isoflavonoids, flavonoids, stilbenes, and lignans. Of these, stilbenes are a group of polyphenols containing bioactive molecules in the plant world. (1-3)

Bioactive components found in plant sources are secondary metabolites that have various functions in plant growth and development. In addition to providing plant-specific features such as taste, color, and odor; they also provide protection against bacteria, viruses, fungi and pests. Bioactive components that are generally found in plants as esterified or bound to glycosides; they contain an aromatic ring and at least one hydroxyl (-OH) group. It is estimated that there are more than 30,000 bioactive components that differ from each other by the structural differences of the aromatic rings, the number of -OH groups, the bonds they form with various carbohydrates and organic acids, and many of them take place in our daily diet. (3-5)

RES is a bioactive compound that is a stilbene derivative. It is a phytoalexin naturally synthesized or produced by several plants in response to environmental stress, mechanical injury, pathogen attacks (such as fungi/bacteria), UV-irradiation or ozone exposure. (6,7) It is induced in plants as a part of their defense mechanism. RES was first isolated from the roots of

hellebore (*Veratrum grandiflorum* O. Loes) in the 1940s and later from the roots of *Polygonum cuspidatum*, an herb used in traditional Chinese and Japanese medicine in the 1960s. (1) Discovered in the vine in 1976, it has been shown that RES is produced in huge amount by the vine plant in response to biotic infections, but also by reacting to abiotic stresses. (8)

2.   Chemical Structure and Sources of Resveratrol

RES (3,4',5-trihydroxystilbene) is a natural polyphenol, possessing two phenol rings linked to each other by an ethylene bridge. (1) It is structurally similar to diethylstilbestrol, a synthetic estrogen. (9)

The molecular formula of RES is $C_{14}H_{12}O_3$ and formal chemical name (IUPAC name) of it is (E)-5-(4-hydroxystyryl) benzene-1,3-diol but it is also mentioned by alternative names such as 3,4',5-stilbenetriol, (E)-5-(p-hydroxystyryl) resorcinol. (10) The chemical structure of RES is identified in two isomeric forms, trans- and cis- RES (Figure 1). Of these two known isoforms, trans-RES is more stable than the cis-form. (11) UV light and high pH lead to isomerization of the trans-isoform to the cis isoform. (12) On the other hand, factors such as visible light, high temperatures and low pH are also effective in isomerization from cis- to trans- form. (1,13) The trans-isomer is preferred in most studies due to its availability in nature, stability, and higher biological activity. (10)

trans-RES   cis-RES
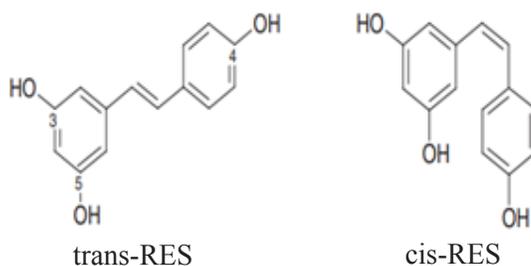
Figure 1: Chemical Structure of RES
(trans- and cis- forms) (Modified from 15)

RES is produced naturally by 72 different plant species including grapevines, pines, and legumes. It is also found in peanuts, soybeans, and pomegranates in high concentrations. (14) RES is present in human diet i.e., in fruits such

as grapes, strawberry, blueberry, lingberry, sparkleberry, cranberry, mulberry, bilberry and in flowers and leaves such as butterfly orchid tree, eucalyptus, spruce, lily, gnetum etc. (6,15)

Grapes and grape-processed products are the most promising sources of RES. (13) RES accumulation in grapes depends on grape variety, genotype, environmental conditions, location, and growing seasons. Although each part of the grape, such as buds, roots and leaves, skins, stems, shoots, bark and seeds, contains varying amounts of RES, the highest concentrations of RES are found in the grape skin. (13,16) This is because *Botrytis cinerea* infection in grapes leads to the exclusive synthesis of RES in the leaf epidermis and grape skins. (14,13) Although not as much as the grape skin, red wine can be considered as one of the most concentrated food sources of RES due to the grape parts used in its production. (17,18)

Today, 92 new RES compounds, including 39 dimers, 23 trimers, 13 tetramers, 6 RES monomers, 6 hexamers, 4 pentamers and 1 octamer, have been reported from the families Dipterocarpaceae, Paeoniaceae, Vitaceae, Leguminosae, Gnetaceae, Cyperaceae, Polygonaceae, Gramineae and Poaceae. (19) For industrial purposes, RES is also produced by chemical and biotechnological synthesis and sold as a nutritional supplement. (20,21)

3. Resveratrol Biosynthesis in Plants
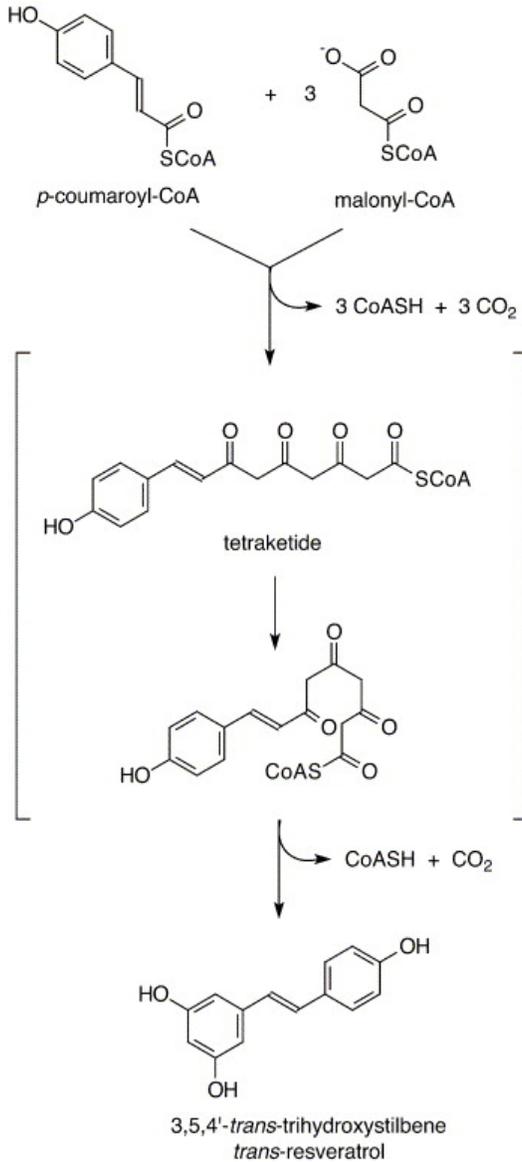
Figure 2: Biosynthetic Pathway of RES (22)

The biosynthesis of RES is catalyzed by the enzyme stilbene synthase. RES consists in the repetitive decarboxylative condensation of a p-coumaroyl residue from p-coumaroyl-CoA with three C2-units from malonyl-CoA (Figure 2). This is followed by conjugation of RES with glycosyl or sulfate residues. (22)

4. Bioavailability of Resveratrol

RES is classified as a Class II compound by the Biopharmaceutical Classification Scheme as it is a low water-soluble natural product with high membrane permeability. This classification provides a theoretical basis to correlate *in vitro* drug dissolution and *in vivo* bioavailability. (23)

Although RES has been confirmed to have beneficial health effects, this compound has specific pharmacokinetic properties that limit its use. (24) RES has interestingly high absorption for a compound with poor aqueous solubility. (15) The extensive metabolism and rapid elimination of RES cause it to exhibit poor bioavailability that hinders therapeutic applications. (24,25) Therefore, many RES derivatives with therapeutic potential have been synthesized by esterification processes, such as methoxylated, hydroxylated and halogenated derivatives. (26)

Food products contain cis- and trans- isoforms of RES, mostly in glycosylated form, called piceids. Glycosylation prevents enzymatic oxidation, thereby the glycosylated RES analogs exhibit stronger bioactivity with increased overall stability and bioavailability. (15) Similarly, pterostilbene, a naturally methoxylated RES analog, has a higher pro-lipophilicity than RES and has a higher bioavailability. This allows it to show stronger bioactivity. Again, piceatannol, which has an extra hydroxyl group, is reported to have stronger anti-inflammatory, immunomodulatory, anti-proliferative, etc. effects (Figure 3). (1,22)

Based on the fact that a small increase in the solubility of RES will significantly increase its bioavailability; delivery systems facilitating its rapid absorption have been proposed in order to increase its plasma concentration. Upon this, to improve the poor bioavailability of RES, various methodological approaches have been developed such as lipid nanocarriers or embedding in liposomes, emulsions, micelles, polymeric nanoparticles, and RES encapsulation in nanocrystals. (12)

5. Metabolism of Resveratrol (Absorption, Transport and Excretion)

RES's low solubility (<0.05 mg/mL) due to its chemical structure affects its absorption. (16) After oral administration, RES is absorbed mainly from the jejunum and some from the ileum by passive diffusion or membrane transporters and released into the bloodstream. (27) While still in the gut, RES is metabolized by glucuronidation of phenolic groups and sulfate conjugation as

well as hydrogenation of the aliphatic double bond. (12) RES, which can cross the intestinal epithelium, is only 6% of native RES and its conjugates that can reach the intestines. (28)
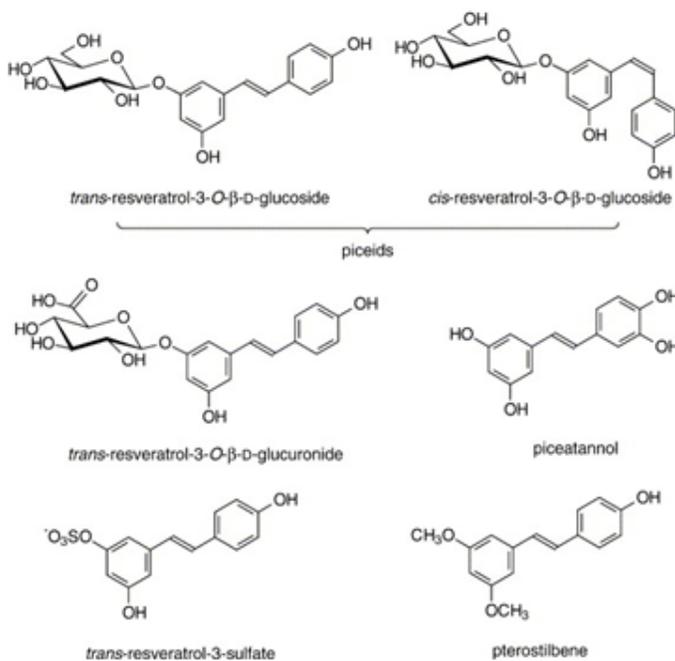

Figure 3: Most Common Conjugates And
Analogs of RES (modified from 22)

Trans-piseid is deglycosylated in the small intestine by the enzymes; lactase phlorizine hydrolase and cytosolic-β-glucosidase. Trans-RES is formed by deglycosylation from trans-piseid. Trans-RES is further metabolized in enterocytes to form glucuronide conjugate. Glucuroconjugate is released from enterocytes into the intestinal cavity. (29)

RES and its metabolites are filtered from the blood by the liver and gallbladder and excreted into the intestines with bile. It is then reabsorbed. RES is rapidly metabolized in the liver; it binds to lipoproteins and albumin in plasma and thus can easily enter cells. (30) It is reported that the majority of plasma RES metabolites are RES-3-O-sulfate, RES-4'-O-glucuronide, and RES-3-O-glucuronide, all of which have little bioactivity. (12) Hydrophilic conjugation of RES is a factor that facilitates its passage into the blood, distribution, and

excretion in the body. With a half-life of nine hours, distribution of RES into tissues may take several hours. (25)
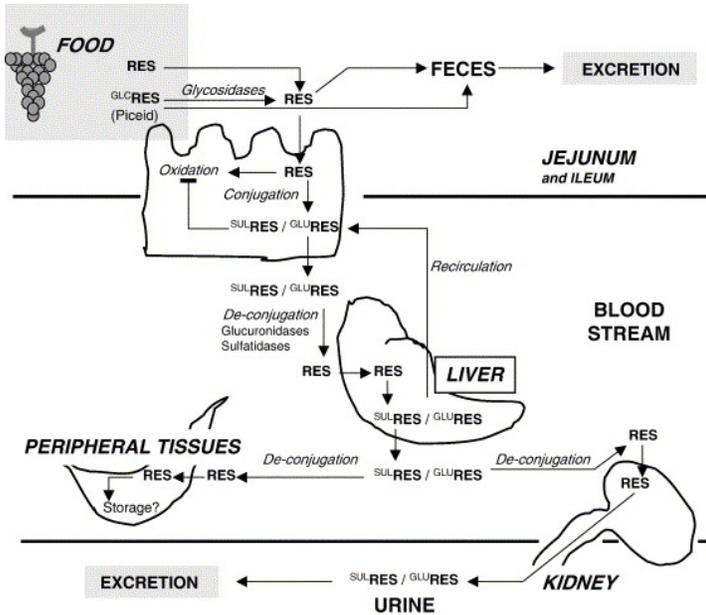
Figure 4: Pathways of Resveratrol Absorption,
Transport, Metabolism, And Excretion (22)

According to animal experiments, renal excretion of RES begins a few hours after ingestion and continues to increase over the next 1-2 hours. (29) The presence in urine of little native (aglycone) RES but high amounts of its conjugates indicates that metabolism of the compound is essential for excretion. (31) Five different metabolites of RES are seen in the urine. These are: RES monosulfate, two isomeric forms of RES-monoglucuronide, monosulphate dihydro-RES and monoglucuronide dihydro-RES. (12) While a small amount of RES is rapidly metabolized, it can accumulate in organs such as the heart, liver and kidneys when taken above a certain dose. (32) The metabolism of RES is shown in Figure 4. (22)

6. Therapeutic Effects of Resveratrol

RES, which is the focus of many cell culture, animal and human studies, is an all-purpose compound believed to be effective in promoting health and preventing/treating chronic diseases. Therefore, it has been included in many *in vivo* and *in vitro* studies. The research on RES started in 1992, through the

"French paradox" which describes the low incidence of coronary heart disease among French people despite a high-fat diet. Many researchers have suggested that moderate consumption of red wine, the richest source of RES, explains this apparent contradiction. (12,14,33)

Nowadays, RES is considered as a bioactive molecule with potential beneficial effects on health due to its pharmacological properties and lack of harmful effects. (34) RES is one of the best known phytophenols exhibiting pleiotropic properties. Besides being a phytoalexin, it provides numerous beneficial effects to animals and humans by maintaining the homeostasis of a wide variety of organs and tissues. (18)

The use of RES as a nutraceutical compound is noteworthy because of its therapeutic effects. (35) This natural polyphenol has numerous pharmacological effects, including anti-inflammatory, hepatoprotective, anti-diabetic, anti-cancer, antioxidant, cardioprotective and anti-adipogenic. (33,36-41) As a pharmacological tool, RES has a wide range of molecular targets. These observed effects are believed to be due to its simultaneous effect on multiple targets. (33) RES generally achieves these effects by modulating enzymes belonging to various classes such as kinases, lipoxygenases, cyclooxygenases and sirtuins and acting as a potent free radical scavenger. (42,43)

RES is currently used to treat cancer, slow aging, neurodegenerative and cardiovascular diseases, antiviral therapies, inflammation, platelet aggregation, and a host of other disorders. (13) According to new evidence, RES also helps maintain genome stability. It contributes to the inhibition of cancer caused by genomic instability by activating the repair of DNA double-strand break. (37)

As an antiapoptotic agent, RES is reported to provide protection from various diseases such as myocardial ischemic reperfusion injury, atherosclerosis, ventricular arrhythmias and cerebral ischemic. Also, RES, can be used in combination with other chemotherapeutic drugs and radiation therapy to increase their therapeutic efficacy while limiting the adverse side effects associated with chemotherapy and radiotherapy. (44)

RES is also famous for its important business applications. (6) Not only the pharmaceutical industries, but also companies investing in cosmetics and food additives have shown increasing interest in RES. For example, due to its potential as a topical anti-aging compound, RES has gained popularity in dermatology applications as a cosmeceutical to improve skin health or there are many nutraceuticals commonly available over-the-counter. (33)

7.   Some Biological Activities of Resveratrol

7.1 Antioxidant Activity

Oxygen is an essential element for life. Oxygen causes the formation of many reactive oxygen species (ROS) during the energy generation process. ROS at physiological concentrations are necessary and have many beneficial effects. They play important roles in defense functions such as killing cancer cells, detoxification of xenobiotics, and moreover, cellular signal transmission mechanisms. If ROS are not effectively scavenged by cellular constituents, they can stimulate free radical chain reactions and initiate peroxidation of membrane lipids, leading to accumulation of lipid peroxides. Then cellular biomolecules such as proteins, lipids and nucleic acids are damaged and eventually diseases arise. (45)

Aerobic organisms have antioxidant defense systems to remove or repair damaged molecules. Antioxidant compounds can slow down the lipid peroxidation process and scavenge free radicals. ROS levels higher than physiological concentrations cause imbalance between oxidative and antioxidative processes, named oxidative stress. Oxidative stress is a factor that induces a number of diseases such as atherosclerosis, cardiovascular diseases, diabetes, inflammation, aging, and neurological diseases. (46)

Polyphenols contain a broad class of antioxidants, including flavonoids, phenolic acids, lignans, and stilbenes. RES, a stilbene derivative, also has many biological activities, but the best known is its capacity to act as a potent antioxidant. (22) RES concentration and cell type are factors that determine either it will act as an antioxidant or a prooxidant. At lower concentration (5 mM–10 mM) RES functions as an antioxidant, while at higher concentration it acts as a prooxidant. (15)

RES inhibits lipid peroxidation due to its hydrogen donor feature. (47) RES can either increase the activity of antioxidant enzymes or act as a scavenger of free radicals. RES is known to act as a scavenger of hydroxyl, superoxide and other radicals. Thus, it prevents DNA lesions and lipid peroxidation in cell membranes. (48) It has been shown that RES can maintain the concentration of intracellular antioxidants in biological systems. It is reported that RES significantly reduces the oxidation of thiol groups in the proteins of human platelets, and also increases the concentration of some antioxidant enzymes such as glutathione peroxidase, glutathione S-transferase and glutathione reductase. (49,50)

In vitro studies have shown that RES is an effective antioxidant; but it is not clear whether it has this property *in vivo*. (47)

7.2 Anti-Inflammatory Activity

Inflammation, a vital part of the human immune system, is the body's immediate response to harmful stimuli such as pathogens, chemicals, or tissue and cell damage caused by physical injuries. Acute inflammation is a short-term response, in which leukocytes infiltrate the damaged area to repair the tissue and remove the stimulus, usually resulting in healing. In contrast, chronic inflammation is a prolonged, dysregulated, and maladaptive response. (51) Nowadays, it is widely believed that chronic inflammation has an important role in the pathogenesis of various chronic diseases, including metabolic, cardiovascular, pulmonary, and neurological disorders. (52,53) In addition, studies have shown an association between inflammation and allergy, arthritis, certain types of cancer. (51,54)

Although inflammatory response processes vary depending on the nature of the stimulus and its location in the body, they share a common mechanism. In this process, receptors on the cell surface recognize harmful stimuli; inflammatory pathways are activated; inflammatory markers are released; inflammatory cells are recruited, and ultimately target tissues are affected. This complex sequence of events results in pain, heat, redness, swelling, and eventual loss of function, which are the primary signs of inflammation. (33)

Stilbenoids including RES are polyphenols with acidic and amphiphilic characters with anti-inflammatory activity. Studies reported the ability of RES to reduce the secretion and expression of inflammatory factors. (55) The anti-inflammatory effects of RES are predominantly due to inhibition of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and 5-lipoxygenase activities, resulting in suppression of prostaglandins, thromboxanes, and leukotriene formation. (12,56) It has also been observed that this compound attenuates macrophage/mast cell-derived proinflammatory factors such as platelet activating factor (PAF), tumor necrosis factor-α (TNF-α) and histamine. (57,58)

An important function of RES is to inhibit the production of inflammatory factors through activation of Sirtuin 1 (SIRT1). SIRT1 is an important deacetylase involved in numerous molecular events. Activation of SIRT1 by RES reduces the expression of NF-κB-mediated inflammatory factors such as IL-1β, IL-6, metalloproteases (MMPs), TNF-α, COX-2. (13,57)
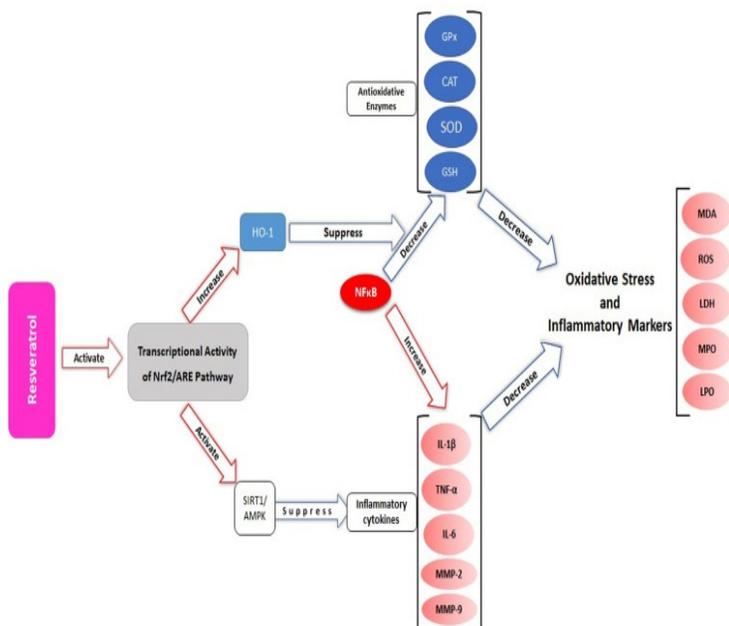
Figure 5: RES, Oxidative Stress And Inflammation (35)

Generally as a pharmacological tool that modulates enzymes belonging to several classes including kinases, lipoxygenases, cyclooxygenases and sirtuins, RES has a wide range of molecular targets and the observed effects are believed to result from its simultaneous action on multiple targets (Figure 5). (42)

7.3   Immunomodulatory Activity

RES modulates innate and adaptive immunity by interacting with several molecular targets. (59) At the molecular level, RES targets cellular processes such as gluconeogenesis, lipid metabolism, mitochondrial biogenesis, angiogenesis, and apoptosis, as well as sirtuin, adenosine monophosphate kinase, inflammatory cytokines, antioxidant enzymes. (57) RES can reduce cytokine production, limit neutrophil activity, change the expression of adhesion molecules, suppress the toll-like receptor (TLR4) and expression of pro-inflammatory genes'. (13)

Regarding the immune system, RES participates in the activation of macrophage, T cell and natural killer (NK). (59) Effects of RES pertaining to immunity are the result of its ability to remove ROS, to inhibit COX, and to activate many anti-inflammatory pathways, including SIRT1. SIRT1 disrupts the TLR4/NF-κB/STAT signal which in turn decreases cytokines production from inactivated immune cells. (58)

RES modulates immunity by interfering with immune cell regulation, synthesis of proinflammatory cytokines, and gene expression. The antioxidant activity of RES and the ability to inhibit enzymes involved in the production of eicosanoids contribute to its anti-inflammation properties. The effects of RES on the immune system are important for different autoimmune and chronic inflammatory diseases. (57)

RES pathways in immune function; RES activates SIRT1, this decreases NF-κB induced expression of TNF-α, IL-1β, IL-6, MMPs, and COX-2. Cyclic adenosine monophosphate (cAMP) levels trigger protein kinase A , which activates SIRT1. AMP-activated protein kinase (AMPK) controls the activity of SIRT1 by regulating the cellular levels of nicotinamide adenine dinucleotide (NAD+). An increase of NAD+ levels induces SIRT1 activation, which promotes deacetylation and activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). (57)

On the whole it is stated that RES modifies cell morphology, gene expression, ligand-receptor interactions, signaling pathways and foam cell formation. (60) In addition, RES is reported to modulate the immune response by affecting cellular prostaglandin E2 (PGE2) levels, which play an important role in regulating the immune response. (61) The cell-specific effect of RES on interleukin production is another important function of RES. (62) However, RES only affects immune cells for a limited time due to its short half-life in the blood. (25)

7.4   Cardioprotective Activity

Cardiovascular diseases (CVD) are complex multifactorial diseases that are one of the main causes of morbidity and mortality in the world and are affected by environmental and genetic factors. CVD, which is defined as diseases that mainly affect the heart and blood vessels; includes diseases such as coronary heart disease, hypertension, cerebrovascular disease, congenital heart disease, peripheral arterial disease, heart failure and rheumatism. (63)

Inflammation and oxidative stress play an important role in the development of cardiovascular diseases. (64) The cardiovascular system is most affected by ROS. It is well known that lipopolysaccharides disrupt redox homeostasis, increase oxidative stress, and reduce antioxidant enzymes in the heart. (35) RES has the ability to potentially alleviate oxidative stress-induced cardiovascular disease by suppressing the high production of reactive oxygen species (ROS) that cause tissue damage, inducing antioxidant defense mechanisms, and limiting lipid peroxidation. (65)

Atherosclerosis is the cause of cardiovascular diseases. It is characterized by many micro- and macrovascular complications, including chronic inflammation, oxidative stress, and lipid deposition in the arterial intima. (64) This is associated with endothelial dysfunction, intracellular oxidative stress, and consequent disruptions in the NO pathway, a potent vasodilator that improves tissue perfusion. (66) Physiological roles of NO are to regulate vasodilation, decrease platelet aggregation, decrease leukocyte uptake, and smooth muscle cell proliferation. RES may promise potential benefits in CVDs, both as an antioxidant and a regulator of NO metabolism. RES also improved heart function, endothelial function, red blood cell deformability, and decreased serum LDL-cholesterol level and platelet aggregation. (25)

Numerous preclinical studies in animal models attempt to explain the beneficial effects of RES in CVDs through potential multiple molecular targets. Inhibition of prohypertrophic signaling molecules, enhanced myocardial Ca^{2+} handling, phosphorylation of prosurvival (Akt-1, GSK-3), and stress signaling (MKP-1) pathways, an enhanced expression of the antioxidant mitochondrial enzyme Mn-superoxide dismutase (SOD2), an increase in the antioxidant glutathione levels, decreases TNFα production, activations of SIRT-1, COX-2, endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), nuclear erythroid 2-related factor 2 (Nrf2) and antioxidant response element (ARE) are some of the molecular mechanisms of RES action. (13,1) The global action of RES thus results in a decrease of endothelial apoptosis, endothelial activation, and vascular inflammation, and improves the endothelial function. (7)

7.5 *Neuroprotective Activity*

Inflammation, which triggers complex molecular and cellular responses to maintain normal body physiology, damages surrounding healthy tissues if it becomes excessive or chronic. (67) It is thought to play an active role in neurological disorders as well. Interactions of the central nervous system (CNS) with the immune system and neuroinflammation are accepted as aggravating factors in many CNS pathologies. These neurodegenerative diseases include diseases such as Amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD), and Multiple sclerosis (MS). (33)

Neuroinflammation is a complex event involving the activation of microglia, astrocytes and endothelial cells that participate in the blood brain barrier (BBB), infiltration of plasma proteins and immune system cells into brain tissue, and interaction of inflammation-related mediators with brain tissue.

Stimulation of microglia and astrocytes causes the release of inflammatory mediators and cytokines. Activation of the NF-κB pathway in glia cells increases the production of pro-inflammatory cytokines, on the other hand, it produces the pro-inflammatory enzymes, iNOS, COX2 and NADPH oxidase (NOX). (68) Activated NOX enzymes are the main agents in the formation of ROS, and in the brain, especially NOX2 is important in the pathophysiology of Parkinson's, Alzheimer's, amyotrophic lateral sclerosis, and epilepsy. (69)

Increased oxidative stress is associated with neuronal cell death during the pathogenesis of multiple chronic neurodegenerative diseases. (69) An intermediary molecule called the antioxidant responsive element ('Antioxidant Responsive Element'; ARE) accompanies the transcriptional activation of protective genes. ARE is activated by binding of Nrf-2. It is thought that activation of the Nrf-2-ARE pathway protects cells from oxidative stress-induced cell death and therefore this may be a new neuroprotective pathway that provides resistance to these processes. (70)

RES can inhibit pro-inflammatory signaling pathways such as NFκB and p38 mitogen-activated protein kinase (p38 MAPK), by a mechanism dependent on adenosine receptors. (35) It has also been reported that SIRT1, Nrf2, heme oxygenase-1 (HO-1), and phosphoinositide 3-kinase (PI3K)/Akt, including trophic factors and protective signaling pathways, are positively modulated by RES. Adenosine receptors as a new target for RES-mediated glioprotection. (71,72)

On the whole, some researchers have pointed to a potential neuroprotective activity for RES based on its beneficial effects in various models of brain injury. (36) It has been reported that inhibition of synthesis/release of proinflammatory mediators, modification of eicosanoid synthesis, inhibition of activated immune cells and inflammatory enzymes such as iNOS, COX-2, inhibitory effects on NF-κB or AP1 signaling pathways may be possible mechanisms. (58)

7.6 Antitumor Activity

Cancer disease is an abnormal cell proliferation that cannot be controlled or stopped. Cancer development is traditionally a multistage process involving molecular and cellular changes that can be divided into initiation, promotion, and progression. (33)

Inflammation is recognized as a critical component of tumor progression. RES can act on inflammatory signals involving a complex interaction between

oncogenic and tumor suppressor transcription factors with its anti-inflammatory, antioxidant, pro-apoptosis, and anti-proliferative effects. (73)

Today many *in vitro* and *in vivo* studies confirm that RES can inhibit all stages of carcinogenesis; provides evidence that it not only acts as a chemopreventive agent but also exhibits chemotherapeutic properties. (37) The role of RES in the prevention of various human cancers such as breast, uterus, blood, kidney, liver cervix, eye, bladder, thyroid, esophagus, prostate, brain, lung, skin, stomach, colon, head, neck, bone, ovary has been studied. (13)

NF-κB is a transcription factor found in all cell types that regulates inflammation and immune responses. Several in vitro, preclinical, and clinical studies highlight that NF-kB-dependent gene expression plays an important role in the growth and invasion of various tumors, thus deregulation of NF-κB activity is effective in cancer development. It is accepted that RES is associated with attenuation of NF-κB signaling. Furthermore, it increases the efficacy of chemotherapy by inactivating NF-kB. (74)

It has been reported that RES stimulates Nrf2 activation and thus modulates apoptotic indices (caspase-9, caspase-3, Bax and Bcl-2) and oxidative stress indices (MDA, GSH, GPx, SOD and CAT activities). RES inhibited colon cancer through Nrf2/HO-1 signaling, resulting in a decrease in iNOS, COX-2 and aldose reductase expressions and an increase in glutathione reductase activity. (35)

RES is also a Histone deacetylase inhibitor that exerts its antiproliferative effect by activating cell cycle arrest, inhibition of angiogenesis, inducing apoptosis and autophagy, mitotic cell death and generation of reactive oxygen species that cause oxidative stress in cancer cells. (75) The ability of RES to modulate detoxifying enzymes (Phase I and II) has also been demonstrated in preclinical studies. (35)

In sum, RES functions as an antiapoptotic agent at low doses, while at high doses it exhibits proapoptotic properties in cancer cells. RES is known to participate in all three stages of oncogenesis by affecting various intracellular mediators. Depending on the tumor model, intracellular targets can be NO, tumor suppressor p53, apoptosis regulators, cyclooxygenases, transcription factors, cyclins, calpains, caspases, interleukins, cathepsins, etc. (15)

7.7   *Anti-obesity Activity*

Obesity is one of the most important health problems, which is closely related to many chronic diseases and is defined as excessive/abnormal fat

storage in the body. (27) RES has been found to have effects on metabolic pathways such as adipogenesis, lipogenesis, lipolysis, fatty acid oxidation and thermogenesis. (76)

Adipocytes are derived from mesenchymal stem cells that have the potential to differentiate into myoblasts, osteoblasts, chondroblasts, or adipocytes. The adipocyte life cycle begins with cell shape change, growth arrest, and clonal expansion. A series of complex changes in gene expression then lead to lipid accumulation and subsequent cell death. (77)

Differentiation of preadipocytes and stimulation of metabolic pathways related to lipid metabolism requires expression of several adipocyte-specific genes such as peroxisome proliferator-activated receptor-gamma (PPARg), CCAAT/enhancer binding protein-alpha (C/EBPα), sterol regulatory element binding proteins-1c (SREBP-1c), fatty acid synthase (FAS) lipoprotein lipase (LPL), and hormone sensitive lipase (HSL). (78)

RES can change fat mass by directly affecting biochemical pathways involved in adipogenesis in maturing preadipocytes by down-regulating the expression of adipocyte-specific transcription factors and enzymes. (79) Nevertheless, RES is reported that RES increases the activity of sirtuins, which are the key enzyme family in calorie restriction. Activated SIRT1 induces expression of genes involved in mitochondrial biogenesis and fatty acid oxidation. (80)

Heat production in response to environmental temperature/diet is defined as adaptive or facultative thermogenesis. (81) This process is mediated by uncoupling proteins (UCPs) located in the inner mitochondrial membrane. These proteins suppress oxidative phosphorylation and produce heat by scattering the proton gradient across the membrane. (82) There are few reports in the literature about the effects of RES on this metabolic process. According to these, RES can increase UCP1 in intraspacular brown adipose tissue (IBAT) and UCP3 in skeletal muscle. The idea that these effects are mediated by SIRT-1 is widespread. (76)

On the whole, it can be said that RES has an anti-adipogenic effect. It shows its anti-obesity effect by decreasing adipogenesis and increasing apoptosis. It can be added that it has an effect on lowering body fat with the increase in thermogenesis. RES targets different metabolic pathways involved in triacylglycerol metabolism in white adipose tissue. It also causes a decrease in body fat by inducing de novo lipogenesis inhibition and adipose tissue fatty acid uptake. It cannot induce lipolysis alone but increases lipid mobilization

induced by β-adrenergic agents. In addition, the fact that RES increases mitochondriogenesis and thus fatty acid oxidation in skeletal muscle and liver may contribute to the body fat lowering effect of this molecule (Figure 6). (27,76)
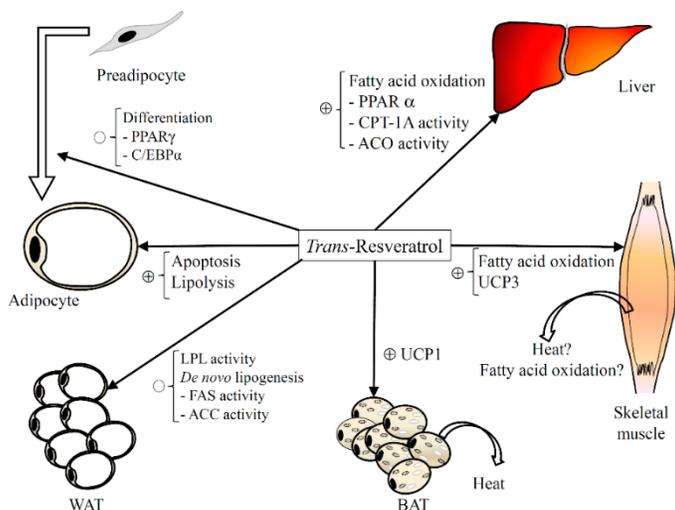
Figure 6: Major Mechanisms Involved in
The Anti-obesogenic Effect of RES (76)

In vivo studies using mostly rodents, it has been reported that RES reduces body fat by suppressing fat accumulation processes and stimulating lipolytic and oxidative pathways, but the effects are seen at particularly high levels due to its low bioavailability. (76)

7.8 Other Biological Activities of Resveratrol

RES also has antimicrobial, anti-diabetic and anti-aging effects besides the antioxidant, anti-inflammatory, immunomodulatory, cardioprotective, neuroprotective, antitumor, anti-obesity effects described above. Vasoprotective, hepatoprotective, respiratory-, reno-, skin- and reproductive protective effects of RES are also reported in the literature. These versatile therapeutic effects of RES, demonstrated in many preclinical and clinical studies, are mostly due to its antioxidant properties. (35)

Nrf2 is a crucial transcription factor. The Nrf2 pathway has a critical function in the modulation of lipid and carbohydrate synthesis, metabolism, and degradation, as well as inflammatory responses and oxidative stress. Translocation of Nrf2 to the nucleus stimulates the activation of genes, including

the antioxidant response element (ARE), which activates the transcription of antioxidant enzymes. (83) Nrf2 regulation is required to maintain homeostasis of cellular function and inhibit the progression of various pathological conditions. It is emphasized that the possible molecular mechanisms underlying these therapeutic effects of RES are somehow related to the modulation of the Nrf2 signaling pathway (Figure 7). (84)
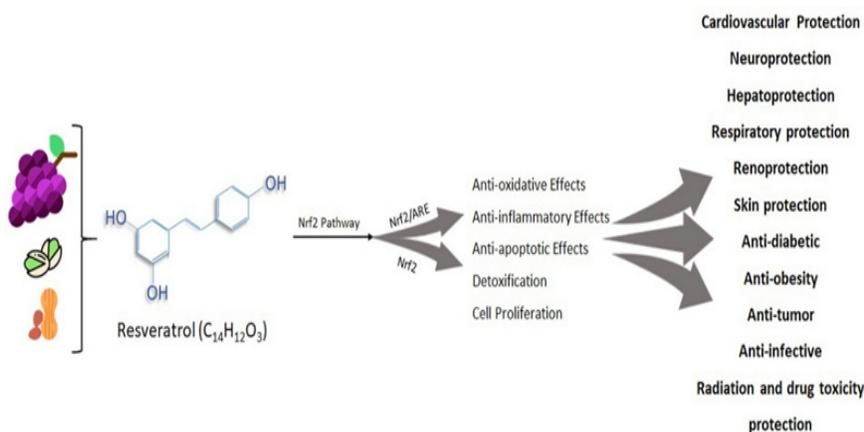
Figure 7: Biological Activities of RES (35

Side Effects, Negative Effects and Limitations of Resveratrol

Natural products have been used as one of the most valuable tools in the production of drugs for the prevention, defence and treatment of diseases, due to their various biological activities. (13)

Recent extensive research has revealed that chronic inflammation may underlie the development of many diseases, including cancer, neurodegenerative, metabolic and cardiovascular diseases. The literature strongly suggests that phytochemicals can interact with multiple targets, altering dysregulated inflammatory pathways and mediators. Therefore, the possible development of affordable, new and safe drugs that can be translated into effective therapies in moleculer and cellular processes is of great importance. (33)

The use of RES for biological purposes dates back hundreds of years. RES is a small, inexpensive molecule that is easy to obtain and functionalize. The absence of debilitating or toxic side effects of RES encourages its possible production as a promising drug. (13)

Having effects such as antioxidant, anti-inflammatory, cardioprotective, neuroprotective or antitumor etc. has caused RES to attract attention as a health-

promoting agent. (12,15) These impressive biological activities of RES, mainly observed *in vitro* and in experimental animals, could not be carried to the clinic due to its poor systemic bioavailability and lack of preclinical toxicological studies. (85) Unfortunately, there are a very few number of studies evaluating the effects of RES with systemic applications. Such studies are needed to know the possible clinical effects of RES and to benefit from them in the clinic. (17)

For instance, a few number of available clinical trials involving RES, are not as promising as preclinical findings regarding its beneficial effects on CVDs. (25) This is partly attributed to the rapid metabolism of RES. However, the concept of the "RES paradox" is proposed, which emphasizes that RES metabolites can exert multiple biological effects despite low plasma concentrations of RES. (86)

Despite its many beneficial effects, the low bioavailability of RES is an obstacle to its meaningful therapeutic application. The poor bioavailability of RES is due to its extensive hepatic gluconuridation and sulfation. In the last decade, various methodological approaches and different synthetic derivatives have been prepared to improve the pharmacokinetic properties of RES. (12,17) It is reported that such analogues may be useful in the prevention and treatment of various diseases. (15)

There are several studies that aimed improving the usability of RES by changing the structural determinant or using RES oligomers or galenic forms (polymeric micelles, nanoparticles etc.). (7) But RES is a phytoalexin and, like many plant-derived products, it shows hormesis. Hormesis is defined as a dose-response relationship that is highly effective at low doses but harmful at higher doses. (88) While developing new technologies such as microencapsulation or nanoparticles to better target tissues, the hormetic action of RES should not be ignored and dose-response studies should be carried out together. (7)

A major disadvantage is that RES is not water soluble but requires organic solvents/oils that are toxic to the environment/human body to dissolve. Also, RES is not stable in organic solvents. Consequently, the water solubility and stability of RES need to be improved for any commercially active formulation. (13)

Further research needs to be performed to know the half-life, tissue distribution and affinity, local metabolism and actions of RES in various tissues of the body. It is suggested that RES may be conjugated with specific monoclonal antibodies and administered *in situ* to target specific tissues or cells. (17)

The effective dose of RES to be used in clinical trials is an important question to be answered. (7) While lower doses of RES may be associated with

health benefits, higher doses have been reported to devastate tumor cells through proapoptotic effects. It has been emphasized that the difference in RES uptake between normal and cancer cells may result from variations in cellular targets and even gene expression in cancer cells. (87) Recent studies implicate that RES displays hormetic action, protecting the cells at a lower dose while killing them at relatively higher doses. (88)

It is noteworthy that RES possibly interacts with drugs, especially CVD drugs. It is frightening to know that there are many RES food supplements on sale at doses much higher than the natural amounts of RES. RES has been shown to inhibit drug-metabolizing enzymes such as cytochrome P450, which is responsible for the phase-I oxidative metabolism of xenobiotics. (89) Modulation of drug metabolizing enzyme activity by RES (1 g/day for four weeks) has been studied in healthy volunteers and was well tolerated.

However, high doses of RES (approximately 1 g/day or more) can lead to interactions with co-administered drugs. (90) It has been reported that these effects may cause safety problems by altering the circulating and/or tissue concentrations of co-administered drugs. In addition, the natural antihypertensive and anticoagulant effects of RES may result in possible interactions with blood pressure, anti-platelet and anticoagulant drugs, and nonsteroidal anti-inflammatory drugs such as aspirin and ibuprofen. (7)

Although a wide variety of doses of RES have been used in various *in vivo* and *in vitro* studies, determining the most effective dosage and route of administration is still critical. (13) In long-term clinical studies, no significant side effects were detected. In fact, RES has been found to be safe and well tolerated at doses up to 5 g/day, either as a single dose or as part of a multi-day dosing schedule. However, it is important to note that these experiments were performed on healthy people and results may differ in sick individuals. It is also difficult to decide which effects can be attributed to RES alone or to both RES and its metabolites, as orally administered RES is rapidly metabolized by the gut microbiota. (13)

Considering the beneficial effects of RES on hypertension, obesity, inflammation, diabetes, and dyslipidemia, RES may constitute an interesting pharmacological approach for the treatment of the metabolic syndrome associated with an increased risk of CVD development. However, the critical thing is not only the poor bioavailability and dose of RES, but also the length of RES therapy and the best time to initiate it. Most studies have also shown that RES is effective when administered for a short time and as a pre-treatment. (91)

Therefore, larger controlled human clinical trials are needed to explore these points and examine the effects of long-term RES supplementation.

It may also be important to elucidate additive/synergistic effects of RES in combination with other treatments. For instance, the application of phytochemicals such as RES in combination with traditional chemotherapeutic preparations in the treatment of malignant diseases will open new perspectives in this field. (15)

Radiotherapy is one of the most effective treatments of cancer patients. Agents that improve the efficiency of radiation killing of cancer cells and prevent the damage to normal cells and tissues are needed. Commercially available RES supplementation can be used efficiently in radioprotection with preventive and/or therapeutic effects. Its antioxidant properties, ability to induce apoptosis and cell cycle arrest, lack of toxicity make RES an attractive candidate for radioprotection of normal cells and prevention of cancer. (6)

8.   Conclusion

RES is a natural compound with various biological applications in the pharmaceutical industry. *In vitro* experiments and animal models show that RES has low toxicity and moreover, it has many beneficial effects on health.

It is important for future clinical research that doses up to 5 g/day for one month are well-tolerated and safe. However, reporting of dose-related mild or moderate side effects in some studies may limit the dose to <1 g/day in clinical trials.

To avoid the non-specific cytotoxic effects of RES, it may be a good alternative to produce better analogues with high bioavailability and specificity. Within this scope, it is important to develop modification and optimization strategies.

Hereby, more research is needed to confirm the multiple effects of RES and its other analogues and to understand their mechanism of action.

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CLINICAL THERAPY OF PREBIOTICS AND PROBIOTICS IN ANIMALS

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1.   Introduction

Prebiotic was firstly described in 1995 by Gibson and Robberfroid like that; it is an indigestible food compound that selectively increases the growing or activition of one or a limited kind of microorganism types in the colon, beneficially effects to the human body and human health (1). However, there has not just been common decision for about the definition of probiotics. Bindels and his friends got the definition of probiotics as "a compound that stimulate the combination and/or activity of internal organ microbiota by metabolization by microorganisms within the bowel and therefore provides a helpful physiological impact on the host" (2). The importance of metagenomic analyzes to investigate animal and human microbiota. Their investigation in point of disease and health has created a exactly new application area in medicine as well as nutrition (3).

The usage of prebiotics and probiotics in pets, farm animals, aquaculture and even plants has become completely common. These practices have high potential at the therapeutic as well as the prophylactic level, and there are many numbers of new technological and microbiologically expensive assays that are slowing progress in the field. The technological defiances for probiotics substantially relate to the necessity for microorganisms to be current at last useable date. Therefore, the usage of bacteria that forms spore has been successfully suggested. Spores have better resistance to ecological situation such as pH changes, humidity and heat. After being digested by animals, it transforms into action vegetative cells (4).

Probiotics are recognized to exert species-certain effects. (5). So, it is important to carefully select types for a specific practice in a special animal. While determining the strains, it may vary according to the animal species (farm animals and pets, etc.) and the desired application. The cost of probiotics may become a problem as the financial opportunities in animal husbandry decline. Also, caution should be exercised in the using of probiotics in pets, primarily for health or animal welfare reasons. As a result of the restrictive side of probiotics is complicated. In people practice, dissimilar countries let several levels of health practice. While no health claims for human application of foods those contain probioticother than yogurt related to lactose intolerance have been approved in the Europian Union (EU), the usage of probiotics in animals is well arranged. Probiotic producers must assure confirmation of the identity, efficacy and safety of the product to be evaluated by the specialists council (6). Probiotic products, when confirmed, are labeled and exchanged as "intestinal flora equalizer" under the category of "zootechnical preservatives" as described by this Low (EC) No 1831/2003 (6). The FDA uses the term "products claimed to contain live microorganisms" (direct-fed microbials) for probioticsthose are used in animal feding (7).

In order to define the advantages of probiotics on wide scale and to advance farmer and consumer acceptability, it is critical to clarify the mechanisms which are underlie the useful atributes. Among these mechanisms; production of antimicrobials, producing of organic acids, decreasing of toxic amines, such as bacteriocins or hydrogen peroxide, or specific enzymes such as proteases, lipases, amylases and glycosidases that can aid digestion may be time-limited during the development of the animal, and effects may often vary by animal species. Therefore, mechanisms specific to animal species should be investigated in detail (8).

1.1. *Polygastric Animals*

Ruminants are the extremely common group of mammals in the world, including about 150 species, both wild and domestic. The economic importance is mostly in cattle, sheep, goat and buffalo breeding (9). Ruminants are uniquely created and, unlike other monogastric mammals, assimilate nutrients from poor quality forage through the digestive system, which includes a four- compartment stomach formed of the reticulum, rumen, abomasum and omasum. In terms of physiologically, every organ performs completely multiple kind processes. In the rumen solid feeds and microbial fermentation of fibers takes place and liquids are

got to the reticulum. The fluids are refined and different nutrients are absorbed in the omasum. In the abomasum, the enzymatic assimilation of the nutrients takes place (10). Whereas the ruminant gut is made up from various sections, giant microbic diversity is ascertained within the rumen, wherever microbic fermentation is performed. The rumen microbiome consists predominantly of bacterial species, as well as archaea, flagellate and ciliary protozoa, fungi, and bacteriophages (11). Since varied physiological parameters of livestock area unit extremely associated with the wealth of assorted microorganism members of the Romanian microbiome, it's necessary to support health and productivity in optimized ruminal fermentation (12). For that, the systematic usage of antibiotics is employed like standard follow in agriculture, still as others, because the use of the helpful falcification of rumen metabolism. However, their common treatment as growth supporters in animal inclose recent years will increase not just for the emergence of antibiotic residues and drug resistant microorganisms in animal product, however conjointly for the health of animals. In recent years, probiotics are wide utilized in placental production within the EU, wherever antibiotic use is totally prohibited (13).

In ruminant cultivation, the employment of probiotics; within the treatment of biological process disorders and reducing enteral pathogens within the equilibration of ruminal pH (14), animal performance and fiber digestibleness (15), premonition of the system (16), rubor therapy (17) has several effects (18). Additionally, a few probiotics, stress or beat infections thanks to the potential to attach mutagenas (19).

Probiotic preparations area unit principally given to animals that brings ruminants within the mouth, directly or in feed. However, probiotic effectiveness could also be vulnerable thanks to oral administration and beat disorders. Therefore, to ensure the stability and viability of probiotics, microencapsulation technology is used in GIT, which ensures the preservation and controlled delivery of the probiotic products (20).

In ruminants, lactic acid bacteria (LAB) are administered like probiotics and DFM. Additionally, lactic acid users such as *Lactobacillus, Enterococcus, Streptococcus, Bifidobacterium, Propionibacterium* and *Megasphaera* elsdenii strains are used, as well as *Bacillus, fibrolytic Prevotella* species and *Escherichia coli* (21). In buffalo and reindeer study, use of cellulolytic Ruminococcus strains ended up with useful modulation of rumen microbiomes (22). While common bacterial probiotics were extremely effective in calves, yeasts and fungi such as *Aspergillus oryzae* and *Saccharomyces cerevisiae*, in orderly, showed better

advantage for adult ruminants (23). Their usage has been shown to positively affect specific bacterial groups and fermentation samples in the rumen (24). In addition, non-living yields obtained from the fermentation of probiotic microorganisms are used efficiently in ruminant animals (25).

The emphasis on identifying probiotics which are used for ruminants is more and more focusing on the target animal's diverse own microorganisms and their use in the animal's digestive tract, which serves as the orginal resource of isolation (26). For example, a comparison of probiotic qualities in isolates which are made from dairy products and in rumen reveals that rumen is more permissive to bile salts and exhibits more limitation towards pathogens (27). These symptom suggest that microorganisms are adapted to a particular ecosystem and may take an important duty in the choosing of new probiotic. Furthermore, the usage of rumen residents as probiotics results in increasing the current useful GIT microbiota, that arises to be a method of gastrointestinal microbiota manipulation than introducing ecosystem-unrelated microbes (28). Therefore, the gastrointestinal tract of ruminant animals is potentially a rich and diverse reservoir for the emergence of new probiotics (29). The improving of high-throughput sequencing techniques reveals the abundance of unculturable bacteria in the rumen ecosystem (30). Recent metagenomic studies which are about the rumen microbiome make a wealth of information not only on the combination and charges of the relevant microbiota, on the orher hand, on its interaction with host and bait (31).

The opinion of appliying bacteriophages to manipulate specific microbial groups in ruminants has also been searched (32). Although phages offer max host certainty, their effective practice makes identity of the bacterial aims in the rumen. One study suggested further testing for probable usage in ruminants as a result of bacteriophage used as a biocontrol for the intestinal pathogen E. coli in rats (33). For an effective therapy method, it is necessary to monitor the growing resistance mechanisms, to use currently isolated phages from the rumen or to develop new phages in the laboratory environment (34). In addition, it is reported that the using of isolated lysines in place of whole bacteriophages may be a hopeful variant, but, more information on rumen viroma should be provided. (35). In addition, further study is necessary to regard the probable risk corporated with the usage of phage and possible contamination of milk and dairy products in lactating ruminants. Probiotics can play a decisive role in reducing rumen methanogenesis. Because it can be achieved by reducing enteric methane emissions, increasing the efficiency of rumen fermentation and increasing the

productivity of animals. The environmental impact of methane from ruminant animals is critical to the sustainability of farm animal as it is accountable for 25% of worldwide methane emissions produced by anthropogenic activities (36). The usage of probiotic acetogenic yeasts and bacteria, especially S. cerevisiae, to reduce methane emissions in the rumen has been researched with hopeful results. Other exciting appearance is the usage of probiotics to specifically control probiotics in the rumen. Because methanogens found in both attached and ciliated protozoal cells have been reported to be in charge of 9-37% of intestinal methane synthesis (37). The comparative relationship between methane emission and rumen protozoa was approved using a meta-analysis approximation (38).

There is also the using of recombinant microorganisms with probiotic qualities in ruminants. The most successful research interets the genetically modified bacterium *Butyrivibrio fibrisolvens*. With this method, a dehalogenase was added for the gene encoding fluoroacetate from the soil species *Moraxella*. (39). The altered organism is able to break down the toxic fluoroacetate found in nutrients. The conclusions are reported to be promising as the microorganism survives in the rumen of cattle and sheep without loss of the relevant gene (40). Last research from different sequencing studies and information bring to lights the plenty of specific to microorganisms in the rumen. Therefore, it seems hard for genetically modified superbugs that are a strain of bacteria that has become resistant to antibiotic drugs, to all colonize the ruminant microbial ecosystem and apply the utilities for which they were designed to the host. Like prebiotics, probiotics, which are non-digestible oligosaccharides, are also efficient in changing the combination and activity of the microbiota in gastrointestinal tract, as they occur practical substrates for the increase of certain advantageous rumen microorganisms (41).

1.2. Monogastric Animals

Monogastric animals are classified in terms of breeding as animals with a simple or single-chamber stomach, mainly poultry, horses and pigs. 13 bacterial phyla have been discovered in the GIT of poultry. The main ones are reported as *Firmicutes, Bacteroidetes, Proteobacteria, Clostridium, Ruminococcus* and *Lactobacillus*. In addition to bacteria, viruses, fungi and methanogenic archaea, are also present in the GIT of poultry (42). While the pigs' intestinal microbiota is mainly composed of bacteria, Thermococci and Methanomicrobia have been identified as a small percentage of archaea (43). The composition and activity

of the intestinal microbiota has a very important effect on animal performance, growth and health (44).

After the years, probiotics have been applied in a number of variety ways in animal husbandry. However, it was demostrated for the first time that *Lactobacillus* strains can improve the growth performance of pigs in the 1960s. The most commonly chosed probiotics in single-stomach animals are yeasts; *S. cerevisiae* and *Saccharomyces boulardii* are bacteria thattake place in the rectum and colon; *Enterococcus spp., Lactobacillus spp., Bacillus spp Pediococcus spp*. are located. The most common usefulness of probiotics in single-stomach animals are increased body weight, reduced risk of diarrhea, increased feed efficiency, and dietary digestibility (45).

There are numerous microorganisms that would be noted viable probiotics, but only a few number of microorganisms meet the useful properties. Various techniques based on immunological, biochemical, molecular biological and microbiological characteristics have been developed to determine and state GIT microbiota from animal intestine and colon. Between, the widespread use of max-efficiency sequencing techniques has revealed a large number of uncultivated bacteria, which has provided a wide descriptionon of the poultry' s enteric microbiota and other animals (46). A full understanding of the genomic functions of the intestinal microbiota and its members, that is, the microbiome, will lead to the development of new or improved strategies for the modulation of targeted probiotic strains and effective microbiota (47).

One species that is widely applied for genetic in poultry is *Lactobacillus reuteri*. Using this species' strains indicating heterologous genes in a poultry feed, a lots of studies have been conducted that have shown hopeful results on animal healths and the growth performance (48). Study is recently pointing on genetically changed strains that can express more than one heterologous gene, since genetic engineering approximations have had well results in poultry (49).

The opinion of using bacteriophages to control or treatment zoonotic bacteria in poultry farming has been based as a cost- effective therapy with important benefits over antibiotics. Instability of poultry gastrointestinal tract which is often caused by wide-spectrum antibiotics, is avoided by using host-specific bacteriophages. These are inherently self-limiting, as they reproduce in the specific bacteria and as long as the bacterium is present (51). Recently, broiler chicks in host-specific bacteriophages to reduce Salmonella and Campylobacter colonization of probiotics alone or in combination with successful research reviewed that demonstrate the ability (52). It is important to ensure intestinal

microbial stability by isolating Campylobacter and Salmonella phages from poultry feces (51). In a study conducted on pigs, it was shown that the using of bacteriophages can be a best strategy against different types of Salmonella (54). In general, it is noted that a phage complex using multiple receptors on the host cell is more powerful in decreasing pathogens than naive phages and makes late the formation of phage resistance (55).

Although the concept of functional food emerged long before, since the end of the 1990s, the scientific evidence available on the usage of prebiotics in animal nutrition for poultry and pigs (58). Most of the research on prebiotics has been conducted in poultry, as it is the common studied single-stomach animal. It has been found that prebiotics increase the stool volume of chicken by selectively stimulating beneficial bacteria and regulating the intestinal microbiota by inhibiting unwanted bacteria such as Salmonella (59). The extremely common using prebiotics that are used in single-stomach animals are inulin, (FOS) fructo- oligosaccharides, galacto-oligosaccharides (GOS) and mannan-oligosaccharides (MOS) (59). It has been noted after the using of MOS to reduce the number of Clostridium perfringens in the intestine in poultry and after the use of inulin as a prebiotic to improve the growth performance of ovipositors and broilers. it shows that the effects depend on both dose and diet (60).

2.   *Conclusion*

Probiotics and prebiotics in animals despite the broad applicability and promising results from studies of various, often inconsistent, because the experimental data is obtained repeatability problems arise. The growth environment, the species and breed of animals, the age and physiological condition of the animal, the diet, the content of the probiotic preparation used (eg. the type of microorganism, live culture or lyophilized cells) and its dose appear to seriously affect the results of the use of probiotics in livestock. The application of prebiotics in animal feed is a relatively recent effort, and although the results are promising, many problems need to be solved, such as determining the effectiveness of prebiotics in the routine diet of farm animals. Advanced techniques such as next generation sequencing, animal mikrobiyota can be very useful to verify the effect of any prebiotic, while at the same time, immunological studies in livestock prebiotic future directions in the intestinal epithelium and changes in the quality of livestock products should focus on. As a result, extensive research is needed for the reliable and viable use of probiotics and prebiotics in the production of ruminants.

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DIETARY POLYPHENOLS: STRUCTURES AND BIOACTIVITIES

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1. Introduction

Plants, which are rich sources of phytochemicals, are very important for human nutrition and the development of new herbal medicines. Polyphenols are a substantial group of phytochemicals. (1) In recent years, researchers and food manufacturers have become increasingly interested in polyphenols due to their potential bioactive effects.

Polyphenols, which are found in more than 8000 species in nature, are secondary metabolites of plants. Secondary metabolites are organic compounds produced by plants, whose primary functions have not yet been discovered. (2,3) Although they are not directly relevant to the growth, development, and reproduction of the organism, they play an important role in the attraction of insects and animals, dispersal of seeds, and/or plant defense against ultraviolet radiation or aggression of pathogens. (4-6) Phenolic acids and flavonoids are the most valuable groups of plant secondary metabolites. (7)

They are used in medicines, flavors, pigments, and perfumes, and they are an important part of the human diet. (5,8) Phenolic acids and flavonoids also act as reducing agents, and free radical scavengers. They have antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory activities. (9) In this respect, dietary phenolics play an important role in the prevention of many diseases. (10)

Polyphenols are found in various amounts in fruit, vegetables, grains, and beverages such as coffee, cocoa, and tea. (11) The consumption of foods rich in polyphenols is effective in preventing various metabolic diseases associated with oxidative stress such as obesity, diabetes, cancer, cardiovascular disease, and neurodegenerative diseases. (12,13)

2.   Classification of Polyphenols

Polyphenols are characterized as compounds with phenolic structural properties and include many subgroups. Polyphenols are divided into subcategories such as phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (anthocyanidins, anthocyanins, flavanols, isoflavones, flavonols, flavanones, flavones, and flavanonols/dihydroflavonols), stilbenes (resveratrol, piceatannol), lignans (sesamol, pinoresinol, sinol), tannins (hydrolyzable, non-hydrolyzable, and condensed tannins) and coumarins (7-hydroxycoumarin, 7-methoxycoumarin). (9,14,15) (Figure 1). Structural differences within each group result from the number and arrangement of hydroxyl groups and their degree of alkylation and/or glycosylation. (16)
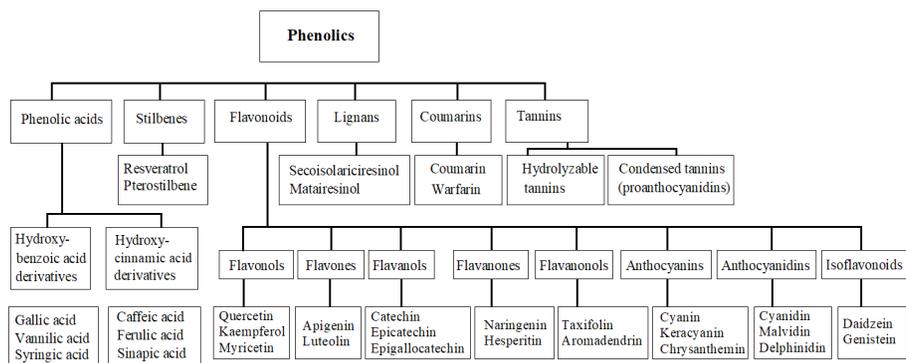
Figure 1. Classification of Dietary Phenolics (17,18)

3.   Chemical Structures and Bioactive Properties of Polyphenols

Plant-based foods naturally contain polyphenols. Polyphenols are potential natural sources of bioactive compounds in plants. Bioactive compounds are phytochemicals that play a role in protecting human health against chronic degenerative diseases. (19) Bioactive components taken into the body with regular consumption of foods rich in polyphenols may help reduce the incidence of liver disorders, various cancers, cardiovascular diseases, obesity, and diabetes. (9)

These compounds have various complex structures. The endless combinations of functional groups (hydroxyls, alcohols, aldehydes, alkyls, benzyl rings, and steroids) in polyphenols provide the occurrence of plant compounds with their unique properties. (20)

Polyphenols are divided into some classes according to their source of origins, biological functions, and chemical structures. The most common classification indicates that phenolics are formed of two essential groups: flavonoids and non-flavonoid compounds. (21) (Table 1). Non-flavonoid compounds are phenolic acids, stilbenes, lignans, coumarins, and tannins. On the other hand, flavonoids can be classified into subgroups, namely anthocyanidins, anthocyanins, flavanols, isoflavones, flavonols, flavanones, flavones, and flavanonols. (Figure 1). The structural diversity of flavonoid molecules results from variations in the hydroxylation pattern and oxidation state. (13,15,22)

3.1. *Flavonoids*

Dietary phenolics or polyphenols are one of many natural product groups. Currently, approximately 4000 flavonoids have been identified out of approximately 8000 types of polyphenols found in the literature, and flavonoids constitute two-thirds of the polyphenols in the diet. (23)

Flavonoids are polyphenolic compounds that have two benzene rings connected by three carbon bridges (C6–C3–C6 skeleton). They are synthesized during plant metabolism and are bioactive compounds found in many commonly consumed foods. (24) They are mainly divided into two classes: (i) anthocyanins (the glycosylated derivatives of anthocyanidins found in colorful flowers and fruits); (ii) anthoxanthins (a group of colorless compounds including flavones, flavans, flavonols, isoflavones, and their glycosides) In the present literature, flavonoids divided into subgroups indicated in Figure 1: Flavonols, flavons, flavanols, flavanons, anthocyanins, anthocyanidins, and isoflavonoids. (17,25)

Flavonoids are largely attributed to their antioxidant properties in plasma, they can also protect cells from various damage. (26) The biological activities of flavonoids, including antioxidant activity, depending on both structural differences and glycosylation patterns. (2)

In meta-analysis studies, higher consumption of flavonoid-rich foods has been associated with reduced death rates from cancer, diabetes, and cardiovascular disease. (27) Certain fruits and vegetables such as blueberries, strawberries, apples, spinach, peppers, and onions are considered rich sources of flavonoids. It has been reported that those who consume these foods frequently

have a lower risk of death than those who do not. (28,29) Many of the beneficial effects of these foods have been attributed to their high content of flavonoids, which have been shown to improve nitric oxide homeostasis and endothelial function and reduce platelet aggregation and oxidative stress. Flavonoids are also effective in inactivating carcinogens, inducing antiproliferation, cell cycle arrest, and apoptosis, and inhibiting angiogenesis. (26,29) In addition, the beneficial effects of drugs are mainly attributed to the presence of flavonoids and associated with their antioxidant activities. (30)

Flavonols (for example, quercetin, kaempferol, and myricetin) are the most abundant flavonoids in plant foods. They are mostly found in leafy vegetables, apples, onions, broccoli, and fruits. Flavones (for example, apigenin and luteolin) and anthocyanidins are found in comparatively small amounts in grains, and herbs. Anthocyanins belong to the group of flavonoids, a subclass of polyphenols. It is known that there are more than 600 anthocyanins in nature. These natural compounds are widely placed in the human diet. They are especially abundant in red, blue, or purple fruits and vegetables. Catechins (e.g., catechin and epicatechin) are abundant in tea, apples, grapes, and chocolate. Flavonones (e.g., naringenin and hesperetin) are predominantly found in citrus and fruit juices. Isoflavones (e.g., daidzein and genistein) are mainly found in soybean and soy-based products. (31) Among them, anthocyanins are the most important group of water-soluble pigments in the plant kingdom. Orange to red, purple in flowers, fruit (e.g., blackberry, red-black raspberry, blueberry, cherry, blood orange, elderberry, grape), and vegetables (e.g., red lettuce, purple potato, radish, red cabbage, eggplant, red onion). They are responsible for a variety of pigmentation, from orange to red, purple, and blue. (32)

Anthocyanins have been the target of many studies because they contain catechol, pyrogallol, and methoxy groups, which provide scavenging, anti-apoptotic and anti-inflammatory activities in their chemical structures. It is suggested as a dietary supplement to alleviate or reduce certain disorders such as diabetes, cancer, cardiovascular, and neurological pathologies. (33)

Flavonoids have many biological effects such as cancer prevention, free radical scavenging, antimutagenic and antiproliferative properties, regulation of cell signaling and cell cycle, and inhibition of angiogenesis. (34)

3.2. *Phenolic Acids*

The second most important group of phytochemicals are phenolic acids, which make up almost the remaining one-third of dietary polyphenols and are found in bound form in fruits. (35)

Phenolic acids are non- flavonoid polyphenolic compounds in C1–C6 and C3–C6 structures. They can be examined into two essential groups, benzoic acid, and cinnamic acid derivatives. (2) Phenolic acids are abundant in foodstuffs. In fruits and vegetables, phenolic acids are in free form, while in grains and seeds —especially in the bran or peel— phenolic acids are in bound form. They are characterized by a carboxyl group attached to the benzene ring (22,36) Phenolic acids can be classified as hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives. (Figure 1). Hydroxybenzoic acids have a C6-C1 structure. Gallic, p-hydroxybenzoic, protocatechuic, ellagic, vanillic, and syringic acids are examples of hydroxybenzoic acid derivatives. In other respects, hydroxycinnamic acids, have a three-carbon side chain, C6–C3 structure. The most common are p-coumaric, sinapic, caffeic, and ferulic acids. (37)

The major sources of phenolic acids are food and beverage raw materials such as pear, cherry (sweet), grapefruit, blueberry, cranberry, lemon, apple, orange, peach, potato, lettuce, spinach, coffee beans, tea, and coffee. (38,39) For example, ellagic acid, which is abundant in cranberries, strawberries, blueberries, and blackberries, reduces blood pressure and high blood cholesterol, and even reduces skin wrinkles caused by radiation. Another example is gallic acid, found in soybean, mango, and tea, mainly known for its antioxidant effect. (40) Gallic acid has protective benefits for human liver cells. (41) Also, it has several reported bioactivities such as diuretic, antineoplastic, bacteriostatic, antimelanogenic, and antioxidant properties. (42) This molecule exhibits anticancer properties in prostate carcinoma cells. (43) Furthermore, due to its ability to suppress cell viability, proliferation, and angiogenesis in human glioma cells, gallic acid has been recommended for the treatment of brain tumors. (44)

3.3. Stilbenes

Stilbenes are synthesized from cinnamic acid derivatives. Its basic chemical structure has two benzene rings connected by a double bond. They are known for a 1,2-diphenylethylene structure with hydroxyl groups on aromatic rings. They exist as monomers or oligomers. (22) The distribution of stilbenes in the plant kingdom is wide. Most stilbenes in plants act as antifungal phytoalexins compounds that are only synthesized in response to infection or injury. (45) Stilbenes are commonly found in roots, bark, rhizomes, and leaves. However, it is usually found in plants or inedible tissues that are not routinely consumed for food. Major dietary sources of stilbenes are grape, soy, and peanut products. (46) They are also found in almonds, beans, blueberries, cranberries, bilberries,

mulberries, and plums. Dietary intake of stilbene has been related with a reduced risk of mortality as well as the onset of hypertension. (47,48)

One of the most well-known and studied stilbenes is trans-resveratrol, which has a trihydroxystilbene skeleton, found largely in grapes. (25) Roots and rhizomes of Polygonum cuspidatum and Veratrun formosanum contain resveratrol and are used in traditional Chinese medicine to treat various ailments. (46) Resveratrol Although it is known to have a cardioprotective effect, it has been reported to significantly reduce fat mass and significantly increase lean mass. (22,49)

Resveratrol inhibits cellular events associated with tumor initiation, progression, and progression. It prevents the occurrence of free radicals that cause tumor initiation. Within this context, it exhibits anti-cancer properties. Quinone, which can detoxify carcinogens acts as an antimutagen as it induces the reductase enzyme. In addition, it has anti-inflammatory activity and cyclooxygenase It inhibits hydroperoxidase activity, thereby inhibiting the arachidonic pathway leading to the formation of prostaglandins, which can stimulate tumor cell growth and activate carcinogenesis. (50)

The pharmacological properties of stilbenes have inspired the synthesis of drug analogs. For example, tamoxifen with the structure of 1,1,2-triphenylethylene containing the stilbene skeleton has been authenticated as an effective for the prevention and treatment of breast cancer. (51) Another synthetic stilbene, 3′-hydroxystilbene, was found to be much more effective than resveratrol at inhibiting the growth of susceptible and resistant leukemia cells. (52)

3.4. Lignans

Lignans are non-flavonoid phenolic compounds containing the 2,3-dibenzylbutane structure formed by the dimerization of two phenylpropanoid units (C6-C3-C3-C6). Secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol are the most common lignans and are considered phytoestrogens. (45,53) Many plants, such as flaxseed, which is the richest identified source of the secoisolariciresinol precursor, contain high concentrations of lignans. Because lignans are found in most fiber-rich foods, they are a more convenient source of phytoestrogenic compounds for western diets than isoflavones. (54)

The main sources of lignans in the diet are oilseeds (soy, rapeseed, flaxseed, and sesame), whole-grain cereals (wheat, oats, rye, and barley), legumes, various vegetables, and fruits (especially berries), and beverages such as tea and coffee. The richest nutritional source is flaxseed, which contains secoisolariciresinol

and low amounts of matairesinol. In addition, the presence of lignans in dairy products, meat, and fish has been reported. Dietary intake of lignans is relevant for the prevention of possible cancer chemopreventive effects and cardiovascular disease. (22,45,55) A meta-analysis study found that a high dietary intake of lignans may reduce the breast cancer risk of women postmenopausal. (56) Lignans (sesaminol, sesamolin, sesamol, and sesamin) found in sesame seeds are primary functional compounds with beneficial properties for human health. (57) Sesame lignans have been found to have anti-inflammatory, antioxidant, anti-cancer, and antimicrobial properties. Also in sesame oil, tyrosinase, elastase, collagenase, and hyaluronidase can be used in cosmetics due to their bioactivity such as inhibition activity. (58)

3.5. Coumarins

Coumarins are a group of plant-derived polyphenolic compounds and are in the class of non-flavonoids. (Figure1). Coumarins form a large group of natural substances known as secondary metabolites. They are found in more than 150 different plant species belonging to about 30 different families. (59)

Coumarins accumulate in large amounts in fruits (such as citrus fruits), vegetables (such as celery), roots, flowers, and leaves. It is found in lesser amounts in the barks and stems. Coumarins are quite high in vegetables, fruits, seeds, nuts, coffee, and tea. (59,60)

Coumarins chemically belong to the benzopyrone family and consist of fused benzene and α-pyrone rings. They have cytoprotective and modulatory functions as therapeutic potentials for several diseases. (61)

Coumarins are mostly known for their anticoagulant effects. Chemically, warfarin is the active ingredient of anticoagulant drugs used for therapeutic purposes because of coagulation disorder. (23) Coumarin and some of its derivatives are anticoagulants such as warfarin, acenocoumarin, and phenprocoumon, choleretic Vitamin K antagonists, which are armillarisin and hymecromone, and the antibiotic novobiocin, which is a potential bacterial DNA gyrase inhibitor. Among the most studied pharmacological activities of coumarins are antibacterial, antituberculous, antifungal, antiviral, antimutagenic, anti-inflammatory, antithrombotic, anticancer, anticoagulant, and antioxidant. (62)

It has been documented that coumarin derivatives have good antiproliferative activity. (63) Other activities include anti-inflammatory, antithrombotic, antimicrobial, antifungal, antiviral (including anti-HIV), anticonvulsant,

antioxidant, and antitumor activities. (64) Researchers reported that coumarins can also act as kinase inhibitors, sulfatase inhibitors, selective estrogen receptor modulators, especially downregulators, and aromatase inhibitors. (62)

3.6. *Tannins*

Tannins are a water-soluble polyphenol group. They are classified as condensed (also called catechin tannins or proanthocyanidins) and hydrolyzable tannins. (65)

Tannins are yellowish or brownish-colored substances consisting of gallic acid (3,4,5-trihydroxy benzoic acid) derivatives, which are intensely found in plants' leaf tissues, epidermis, bark, and flowers, fruits, and other plant tissues. They are commonly found in complexes with alkaloids, polysaccharides, and proteins. (66) They are components of legumes (beans, etc.), fruits (especially berries), and nuts (hazelnuts, etc.). (22)

Hydrolyzable tannins are classified as gallotanins and ellagitannins according to their structural properties. (25) Punicalagin is an ellagitannin abundant in the peel of the pomegranate and is also found in pomegranate juice. Hydrolyzable tannins are also found in strawberries, mangoes, and nuts. (53)

Proanthocyanidins known as condensed tannins are consists of proanthocyanidin monomers or different flavan-3-ol subunits of catechins. The most common monomeric units are (epi)catechin, (epi)afzelechin, and (epi) gallocatechin. (67)

Nowadays, terrific attention is led to proanthocyanidins and their monomers for their beneficial health effects, including immunomodulatory, anti-inflammatory, anticancer, antioxidant, cardioprotective, and antithrombotic properties. (65) Dietary supplements containing proanthocyanidins include cranberry juice is used for the positive effects on the protection of urinary tract infections, and pine bark extracts are used for the prevention/treatment of a wide variety of chronic diseases. (68) In addition, grape seeds have been reported to reduce heart rate and systolic blood pressure. (69)

Many epidemiological data are showing that tannins are useful in the external treatment of skin inflammation and injuries and dietary intake of tannin can prevent the onset of chronic diseases. (70) Tannins can exert their biological effects in two different ways: locally acting in the gastrointestinal tract and non-absorbable (antioxidant, radical scavenger, antimicrobial, antiviral, antimutagenic, and antinutrient) or absorbable from its colonic fermentation, which can produce systemic effects in various organs. (71)

Tannins demonstrate several pharmacological effects, including antimicrobial, anti-cancer, and cardioprotective properties, as well as antioxidant activity. Also, they have beneficial effects on metabolic disorders and prevent the onset of various diseases related to oxidative stress. (65)

4. Dietary Intake of Polyphenols

Plant foods are consumed in significant quantities as part of the daily diet. Today's society has many unhealthy eating habits. Not only intake of snacks but also inadequate intake of healthy foods triggers a significant nutritional imbalance. Such conditions are the main cause of chronic diseases such as cardiovascular diseases, various types of cancers, hypertension, obesity, and diabetes. (72)

Plants contain health-promoting, biologically active compounds known as polyphenols. Polyphenols are found in almost all plant foods. The protection provided by these dietary foods against diseases has been attributed to the entity of polyphenolic compounds with antioxidant properties. (73) Polyphenols not only have specific functions in plants but may also have health-promoting effects on human metabolism. These substances, which are found in foods and taken with the diet, can act as antioxidants, which are very effective oxygen radical scavengers, thanks to the phenolic groups they contain. (74) In addition, epidemiological studies emphasize that regular consumption of polyphenol-rich foods reduces the incidence of various diseases associated with oxidative stress, such as neurodegenerative diseases, cardiovascular diseases cancer, and diabetes. (37)

Phenolic acids are found in almost all plant-derived foods and are important for the human diet. It has been reported that the average phenolic acid intake in humans is around 200 mg per day, depending on dietary habits and preferences. (41)

Flavonoids are the most abundant polyphenols in the human diet. (Table 1). These compounds are approximately half of the 8,000 natural phenolic compounds found in blackberries, blackcurrants, blueberries, grapes, strawberries, cherries, plums, cranberries, pomegranates, and raspberries. (75,76) By consuming these compounds in the diet (with vegetables, fruits, cereals, etc.), the estimated daily intake values can vary between 50-800 mg. (30).

Table 1: General structures of flavonoids, dietary sources, and phenolic contents.

Flavonoids	Example Compound	Structure*	Dietary Sources and Phenolic content**
Anthocyanidins	Malvidin		Strawberry (500), Black chokeberry (878)
Anthocyanins	Chrysan-themin		Black elderberry (1316), Black currant (595)
Flavanones	Naringenin		Pure blood orange juice (51), pure Grapefruit juice (46),
Flavanols	Catechin		Cocoa powder (3410), Dark chocolate (1589), Hazelnut (495)
Flavanonols	Taxifolin		Red onion (158)
Flavonols	Quercetin		Spinach (119), Shallot (112)
Flavones	Apigenin		Whole-grain common wheat flour (73), Globe artichoke heads (58), Black olive (27)
Isoflavones	Daidzein		Soy flour (466), Roasted soy bean (246),
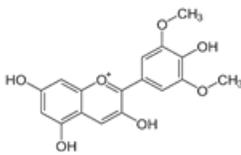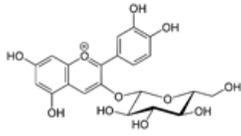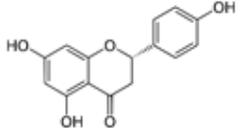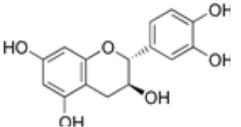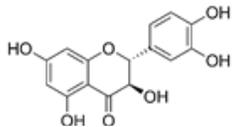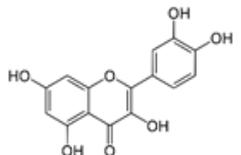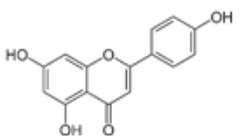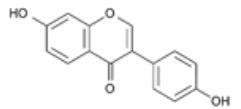
References: (21,77-79)

*The chemical structure formulas of the compounds are from Wikipedia (80)

**(mg/100g or mg/100mL)

Phenolic acids are non-flavonoids that form about one-third of dietary phenols. (Table 2). The remaining two-thirds are flavonoids. (23,25) Phenolic acids are the most prominent class of bioactive chemicals grouped under phenolics found in various plant sources such as fruits, vegetables, spices, grains, and beverages. (81) The most well-known is gallic acid, which can be found in tea, mango, and soy, and is mainly known for its antioxidant effect (40)

Ferulic acid is the major phenolic acid found in cereal grains, which is the main dietary source in the human diet. The ferulic acid content of the wheat grain is 0.8–2.0 g/kg dry weight, which may represent 90% of the total polyphenols. Flaxseed is the richest dietary source of lignans, another group of non-flavonoids, and contains 3.7 g/kg dry weight secoisolariciresinol. (77) The main polyphenols in apples are flavanols and made up 65-85% of the total polyphenol content. Similarly, the major polyphenols in grapes are proanthocyanidins, which are mostly in the skin and seeds. (82)

Blueberries, raspberries, strawberries, cherries, black currants, and purple grapes are the main sources of anthocyanins, compounds responsible for a variety of pigmentation in plants from orange to red, purple, and blue. A 100 g serving of strawberries can provide up to 500 mg of anthocyanins. (79)

Phenols are recommended for diet due to their health effects such as antioxidant, anti-inflammatory, immunomodulatory, anti-allergic, anti-atherogenic, antimicrobial, anti-thrombotic, cardio-protective, anti-cancer, antidiabetic, etc. (81)

Table 2: General structures of non-flavonoids,
dietary sources, and phenolic contents

Non-flavonoids	Example Compound	Structure*	Dietary Sources and Phenolic content**
Phenolic acids (Hydroxybenzoic acid derivative)	Gallic acid		Chestnut (2756), Walnut (1575), Red raspberry (155)
Phenolic acids (Hydroxycinnamic acid derivative)	Caffeic acid		Coffee (267), Red chicory (130)
Stilbenes	Resveratrol		Canada blueberry (656), Red currant (448) Grape (185)
Lignans	Sesamolin		Sesame seed oil (1294), Flaxseed meal (867)
Coumarins	Coumarin		Ceylan cinnamon (9700) Blond orange (279)
Tannins	Tannic acid		Red raspberry (155) Cinnamon (9700) Cranberry (140) Pomegranate juice (204) Dark chocolate (1860)
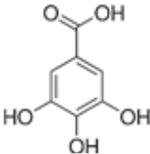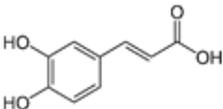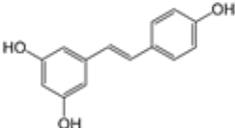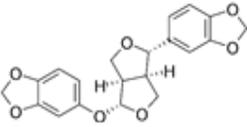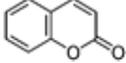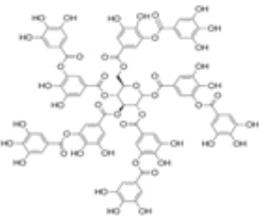
References: (11,83)
*The chemical structure formulas of the compounds are from wikipedia (80)
**(Folin assay; mg/100g or mg/100mL)

Epidemiological studies suggest consuming especially fruits, vegetables, and legumes to prevent diseases. (84,85) Because vegetables are consumed in higher amounts compared to fruits, they can contribute to higher phenolic acid intake in the daily diet. (81)

In recent years, the consumption of naturally based foods has been encouraged, including beans, fruit, legumes, nuts, oils, vegetables, spices, and whole grains. (33) Fruits, vegetables, and other plant-based foods are rich in bioactive phytochemicals to provide desirable health benefits beyond basic nutrition to reduce the risk of developing chronic diseases. (18) Several human cohorts and case-control studies have proven that consuming high levels of fruits and vegetables is related to a lower incidence and mortality rate of some degenerative diseases such as cardiovascular disease, immune dysfunction, and various cancers. (86)

Epidemiological studies and related meta-analyses strongly recommend that the long-term application of diets rich in plant polyphenols protects against cardiovascular diseases, the development of cancer, osteoporosis, neurodegenerative diseases, and diabetes. (45)

5. Conclusion

Phenolic compounds obtained from plant foods are of great interest to nutritionists, food scientists, and consumers because of their role in human health.

Life-long exposure to internal and external factors leads to the occurrence of free radicals in the body through oxidative stress. Free radicals cause aging and many diseases in individuals. The main activity of phenolic compounds is that they are powerful antioxidants that complement and contribute to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress. Thus, they play a supportive role in reducing the risk of disease by strengthening the immune system. Plants are of great importance in human nutrition with their antioxidant effects due to the flavonoids and non-flavonoids they contain.

In recent years, researchers have reported the antioxidant effects of these components as well as their anti-inflammatory, anti-carcinogenic heart-protective, and neuroprotective effects. Because of these beneficial effects on health, polyphenols have been accepted as potential functional nutrients. Besides, epidemiological studies have also reported that polyphenols have powerful effects on the prevention and treatment of diseases such as oxidative stress-induced neurodegenerative diseases, cardiovascular diseases, osteoporosis, diabetes, and various cancers.

More research is needed to propound and support the biological activities of polyphenols, and to characterize their metabolites with specific functional groups, thus meeting their therapeutic, and nutritional needs.

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ACTIVE METABOLITES FROM LICHEN

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1. Introduction

Lichens are a symbiotic association of green algae and/or cyanobacteria and fungal species that benefit each other. This association, which can also be called "lichenified fungi", has characteristics that differ from the organisms that form it morphologically and physiologically (1-3). They are organisms that attract attention due to their different biological and morphological structures and the tasks they undertake in the ecosystem.

Lichens, which are formed by the combination of fungi that make up the mycobiont part and algae and/or cyanobacteria that make up the photobiont part, can continue their lives by photosynthesis, just like in plants (1-3). In the lichen association, the fungus obtains the carbon source it needs to survive from the algae, while the algae obtain the water and minerals to be used in the photosynthetic reactions from the fungus. In addition to this mutual interaction, algae are protected by their fungal partner against adverse conditions (3, 4). In the lichen symbiosis, the mycobiont part is mostly (98%) members of the Ascomycota group (5). The rest consists of members of the Basidiomycota

group. At the same time, the fungus group that the lichen contains is one of the methods used in the classification (identification) of lichen (6, 7).

According to recent studies, it has been reported that there are approximately 20000 different lichen species in the world (2). Lichens, which are rich in diversity, spread in almost all ecosystems. They can spread in a wide range from arctic regions to tropical regions, from mountains to plains, from terrestrial regions (generally included in the terrestrial group), to very arid areas, and even to areas where the seas are tides (3, 8, 9). The environment in which lichens live; rock (on or inside), soil, animal shells, tree trunks, live leaves, as well as rubber, leather, glass, etc. structures can be. It contains some species can adapt to changing environmental conditions and can withstand extreme environmental conditions (extremely hot, dry, salty, nutrient-poor environments, etc.), as well as some species that are highly sensitive to changes in environmental conditions (3). Some species, which are easily affected by changes in environmental conditions, can be used as bioindicators in the detection of pollutants in the environment (air pollution, heavy metals, etc.) (10-13).

The photobiont and mycobiont in the lichen symbiosis act as a system, and as a result, this system synthesizes some metabolites as a product of its metabolism. Some of these synthesized metabolites are synthesized by algae and some by fungi. "Primary (intracellular) metabolites" such as amino acids, proteins, polysaccharides, vitamins and polyols and carotenoids, which are found in many living organisms and are directly related to vital activities, are produced. Metabolites that are not directly related to basic vital activities are called "secondary (extracellular) metabolites" (1, 2, 5).

Secondary metabolites are found extracellularly in the thallus, forming crystals on the surface of algal cells and fungal hyphae. Some of the lichen secondary metabolites are similar to secondary metabolites of organisms such as higher plants or non-lichenified fungi; but the majority of these lichen secondary metabolites are chemical compounds with specific properties (1, 14, 15). While lichen secondary metabolites are mostly synthesized by the mycobiont part, the photobiont is also needed for the necessary synthesis. Studies have revealed that the presence of photobionts is effective in the correct synthesis of many metabolites (14). The fact that the majority secondary metabolites produced by lichen are unique can be explained as follows; although it is seen that fungi isolated from lichen can synthesize metabolites when appropriate conditions are provided in culture medium, these metabolites are different from those produced by symbiotic life (15,16).

Their resistance to environmental conditions and the chance to live in different habitats stem from some unique features of these creatures. Thanks to metabolic products that allow them to withstand harsh environmental conditions, the characteristic features and products of lichen appear. Metabolites also play an important role in many tasks, such as survival, continuity of generation, defense, protection, etc. (17).

These compounds are often called "lichen acids" because they have an acidic character. Lichen acids vary and diversify according to the type of lichen. A lichen species can synthesize a large number of unique metabolites specific to its own species, and the type and amount of metabolite produced may vary according to the environment in which the lichen is located and the stress conditions (5, 15-18).

Although the use of lichen for various purposes (for example, therapeutic in traditional medicine) dates back to ancient times in history, the metabolite varieties of lichens, the production pathways of these molecules, the mechanisms of action have not been fully determined. Recently, with developing technology and methodological methods, scientists have turned to investigate synthesis pathways as the first step in the comprehensive study of these unique bio-active secondary metabolites. This is because the understanding of the synthesis pathways of these chemical molecules sheds light on many unknown points (3).

2.   Biosynthesis Pathways of Lichen Metabolites

The chemical structure of more than 1,000 of the lichen-specific secondary metabolites has been elucidated. As a result of the analysis of lichen acids, it was revealed that their chemical structures were generally polyketoid, quinone, polyphenols, terpenic and aliphatic (12, 18-20).

It has been stated that the types and amounts of lichen acids produced as a result of the studies vary depending on factors such as genetic characteristics, the living species that make up the common life in the lichen structure, the environment in which the lichen is located, stress conditions and their degree (16, 17, 21).

According to the comprehensive studies, the molecule produced by the photobiont species in the lichen structure as a result of photosynthesis and given to the mycobiont partner's differs (1, 3, 19). Products produced according to the type of photobiont in partnership with lichen are given in Figure 1.
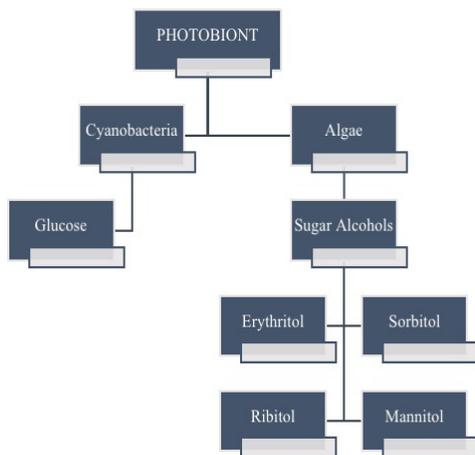
Figure 1. Photosynthesis products, which are produced in lichen symbiont depending on the type of photobiont and given in mycobiont.

The photosynthesis products produced enter into 3 main biosynthesis pathways, basically entering one of the cycles of Glycolysis or Pentose Phosphate after being given in the mycobiont. As a result of these pathways, it forms compounds of various chemical structures. These pathways are illustrated in Figure 2 and Figure 3, and the most important pathway for lichen is the Acetyl Polymalonyl pathway, in which metabolites specific to lichens are synthesized (12).
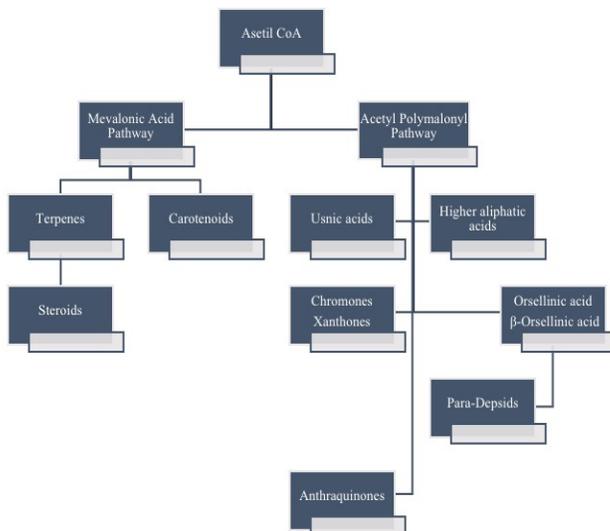
Figure 2. Entry of monosaccharides into the Acetyl Polymalonyl pathway by Mevalonic acid pathway after conversion to Acetyl CoA (Glycolysis cycle).
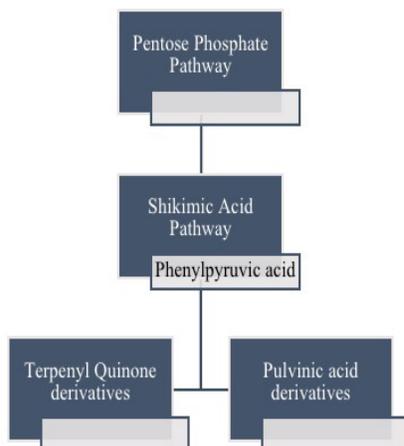
Figure 3. Secondary metabolites produced by the
Pentose phosphate cycle and the Shikimic Acid pathway

The idea that lichen acids are synthesized through these three basic pathways has an important place in the understanding of these secondary metabolites produced. Thanks to these specific substances, lichen has the potential to be used in many areas from medicine to the food industry (2).

2.1. Mevalonic acid pathway

Very few of the lichen acids are produced by this pathway (Figure 2). Terpenes (Monoterpenes, Sesquiterpenes, Diterpenes, Sesterterpenes), carotenoids and terpenoids produced by the entry of Acetyl CoA into this pathway after the reaction of glycolysis are mostly common with those produced in different living things. In addition, triterpenes are synthesized through this pathway and constitute a group frequently encountered in lichen (15, 22).

2.2. Acetyl Polymalonyl (Polyketide) pathway

The vast majority of lichen secondary metabolites are synthesized by this pathway (Figure 2). The first event in the polyketide pathway is the combination of many Malonyl CoA molecules and Acetyl CoA molecules to form the polyketide main chain. After the polyketide main chain is obtained, metabolites with different properties are synthesized thanks to methylation or different ringing patterns (1).

Lichens, thanks to the polyketide pathway; ıt synthesizes a wide variety of chemical classes such as usnic acids, higher aliphatic acids, orsellinic acid

and β-orsellinic acid, anthraquinones and chromones-xanthones, depsids and depsidones (1).

Recent studies have shown that para-depsids are the precursors of some molecules such as meta-depsids, tri-depsids, tetra-depsids, depson, depsidone, diphenyl ether and dibenzofuran (3, 22).

Molecules in the group of para-depsids are synthesized by binding two or three ornisol and ß-ornisol molecules within themselves (by ester, carbon-carbon bonds or ether bonds) (3, 15).

Aromatic compounds synthesized by the acetyl polymalonyl pathway, such as Chromones, Xanthones, Anthraquinones, are regulated by the inner ringing of the polyketide chain. These metabolites also have chemically similar or identical structures to some of the metabolites produced by high plants and non-lichen-forming fungi. Various molecules such as Anthraquinones and Naphtaquinones, which are produced as a result in this biosynthesis pathway, form most pigment substances that are effective in attracting the attention of lichen with their colors in nature as well as the chemical contents they produce (19).

2.3. *Shikimic acid pathway*

This pathway, contrary to above mentioned pathways, synthesizes its own metabolites by combining two phenylpurivates as a result of the pentose phosphate cycle (Figure 3). There are basically two groups of compounds originating from this pathway, these are; Pulvinic acid derivatives and Terpenyl quinone derivatives (15, 19, 22).

3.   Uses of Lichen Secondary Metabolites

Although it is known that the use of lichens dates back to ancient times, it has gained momentum in recent years for people to incorporate them into their lives and try to analyze and group these chemical metabolites in their structure. These products are generally insoluble in water (or dissolved in very small amounts) and can be extracted with organic solvents (2, 5). The ethno-lichenological use of many lichen lichens, as well as the development of new methods and methodologies of lichen metabolites, reveal their biological activity (1, 15-20).

Lichens are creatures that grow very slowly, and scientists who observe in the process have found that they can survive even under very difficult conditions. Their ability to withstand extreme stress conditions has shown that the metabolites they produce have a very important place in the survival

adventure of these creatures. The majority of these secondary metabolites are produced by mycobiont. Another important factor is the increase in lichen secondary metabolites with the increase in some stress conditions. This supports the idea that lichens provide advantages to these chemicals in harsh conditions. In particular, these components are involved in tasks such as adaptation to environmental conditions and defense (4, 23-27).

Lichens have been used in many different fields thanks to their unique pure compounds (22-30). They have a wide range of uses in antimicrobial (1, 4, 28, 31), antiviral (25), anti-inflammatory, antioxidant (4, 28, 31), immunological (2), antitumoral (26, 27, 31), cytotoxic (25-30), insecticidal (2), environmental pollution monitoring and heavy metal accumulation (2, 9-11, 13), cosmetics sunscreens (UV protector) and perfumery (8), food industry, paint industry and many other areas (1). In traditional medicine, lichen has a historical background that dates back to ancient Chinese medicine. When the records were examined, it was seen that lichen had the use of anti-inflammatory, antibiotic, antipyretic, lung tuberculosis treatment, fungal and skin diseases (1, 2, 5).

4.   Conclusions

Today, the mechanisms of resistance to drugs have pushed scientists to find alternative methods that are more effective and have not developed resistance. Lichen secondary metabolites take their place in current studies thanks to their unique structures and rich diversity. With the development of methodologies, elucidation of the structural properties of these chemical compounds is important in alternative therapy.

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ANTICANCEROGENIC EFFECTS OF ELLAGIC ACID AS A NUTRACEUTICAL AGENT

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1.   Introduction

Diseases originating by abnormal cell proliferation that develops due to a malfunction in cell division mechanism during renewal of cells that make up tissues for continuity of life in living things can be generally called as cancerization or cancer. It is known that oxidative stress which occurs as a result of the deterioration of the oxidant-antioxidant balance in organism and resulting from increase in the levels of reactive oxygen species (ROS) is important for the pathogenesis of many diseases including the cancer (1-3). For the prevention of oxidative stress, consumption of intensive herbal sources for antioxidants and researches on action mechanisms of herbal active ingredients continue to be on agenda.

2.   Ellagic Acid

Aromatic chemicals with hydroxyl groups which are among the natural secondary metabolism productions that have important functions for the protection of plants themselves are called phenolic compounds (4, 5). Phenolic

compounds in many nutraceuticals are naturally in fruits such as pomegranate, strawberry, grape or raspberry give a functional food character to plants due to their strong antioxidative activities. Phenolic compounds are grouped under two headings as phenolic acids and flavonoids in plants. The chemical structure of ellagic acid (2,3,7,8-tetrahydroxy [1] benzopyrano[5,4,3-cde][1] benzopyran-5,10-dion) which is a derivative of hydroxybenzoic from phenolic acids including the hydroxyl group and benzene ring is considered to be an ideal feature in reducing free radicals (4, 6, 7). Ellagic acid (EA) is formed as a result of the hydrolysis of tannins and dimeric condensation of hydroxybenzoic acids (6-10). The properties belong to EA such as low solubility in water, being a precursor to molecules that do not dissolve immediately under physiological pH conditions and interacting with intestinal epithelial tissue are important in terms of its potential effects in living things (7, 11). It is also known that EA derivatives have methyl, glucuronyl and sulphate conjugates in plasma and urine (11-12).

Antioxidant, anticancer and anti-inflammatory properties of EA draw attention in both *in vitro* and *in vivo* studies in the field of health (12-15). Ellagic acid is converted to urolithins (urolithin A, B, C, D, M5, M6 and M7 etc.) from its derivates in the gastrointestinal tract by opening one of its two lactones, decarboxylation, and subsequent removal of hydroxyl groups from various positions by the gut microbiota (1, 9, 13) (Figure 1). It has been reported that urolithins C and D as derivates of EA show stronger antioxidative activity than EA and its precursor punicalagin which is concentrated in pomegranate fruit (9). Lee et al. (16) in a study related with microglial cells reported that urolithin B decreased the levels of nitric oxide and ROS, tumor necrosis factor-α and interleukin-6 from proinflammatory cytokines and nuclear factor kappa B (NF-kB) whose activation is important in cancer (11-12).
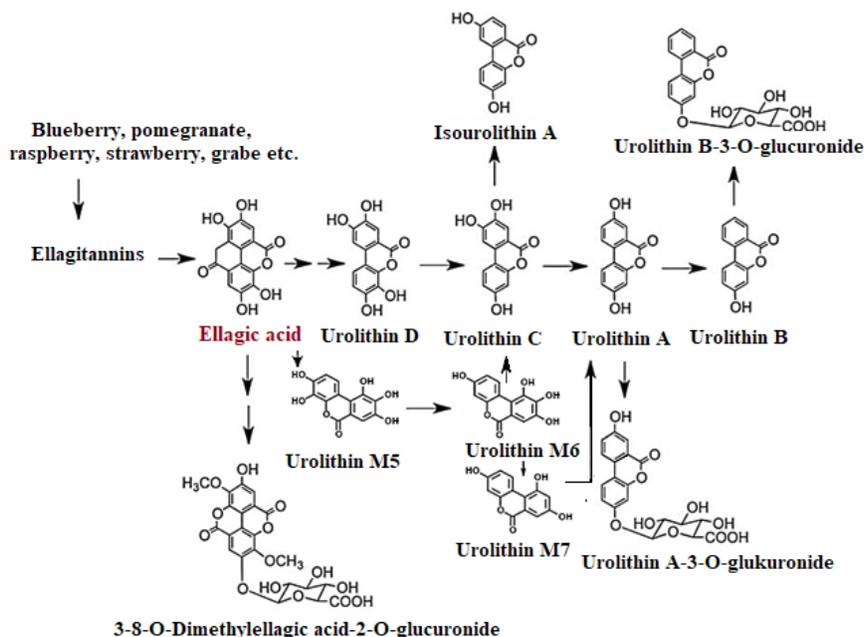
Figure 1. Possible metabolic conversion pathways from
ellagic acid sources to urolithins (9, 12).

2.1. Ellagic Acid and Cancer

It has been reported that EA reduces tumor cell proliferation by breaking the binding of carcinogens to DNA and inducing apoptosis, and also exhibits anticarcinogenic effects by disrupting processes such as inflammation, angiogenesis and drug resistance required for tumor growth and metastasis (17). The results of studies on anticancer properties of EA draw attention as an agent that can be used alone or in combination with chemotherapy drugs to prevent cancer formation, metastasis and even to treat cancer. Therefore, EA can also be considered as a important nutraceutical because it causes biological effects when taken as a dietary supplement in humans and administered at various doses in model animals (3). In addition, the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have generally accepted EA as a safe food or dietary supplement due to its beneficial effects on health (18).

Aticancerogenic activity of EA seems to be valid for various types of cancer. EA and its some derivatives can modify the cellular processes associated with cancer by stopping the cell cycle of cancer cells that are constantly increasing

(14). Studies have shown that EA plays an important role in the treatment of cancer types in various tissues such as colon (19), breast (20) and esophagus (21). Although the targets of cancer treatment differ between cancer stages and types, the strategies developed by inducing apoptosis in studies draw attention. Apoptosis is activated in normal cells by two separate pathways, extrinsic and intrinsic (mitochondrial) following a controlled cell death mechanism. Both pathways lead to caspase cascade activation and may cause to be phagocytosed by macrophages without creating inflammation by converting cells to apoptotic bodies. The method of triggering this apoptotic mechanism which is tried to be explained by caspase-dependent pathways may be one of the popular topics studied in cancer treatment (22).

The clinical effect of EA can be directly evaluated due to the lipophilic and hydrophobic properties of EA and its metabolism to urolithins with different levels of antioxidant activity. According to the log P parameter, EA (LD50 value 1712 mg/kg) is log P positive, so it is hydrophobic and therefore has good binding selectivity in target proteins and is nontoxic (14). Various studies have shown that EA and its derivatives have specific binding domains to block certain proteins involved in cell proliferation in tumorigenesis (22-25). They prevent carcinogen-induced tumorigenesis by directly inhibiting DNA binding of carcinogens such as polycyclic aromatic hydrocarbons and nitrosamines and/ or activating detoxification enzymes including phase I and phase II enzymes (9, 22). They also exert antiproliferative effects by down-regulating insülin like growth factor-II and by arresting cell cycle in G1/S phase, inducing apoptosis, and stimulating expression of tumor suppressor p53 and p21 genes (19, 26). Due to these features, they are seen as a candidate to be a highly effective anticancer agent with very minor changes in their structures. (22, 27).

One of the main targets of ellagitannins is NF-kB, which has dual and different roles in neoplasia. The functions of NF-kB are based on activities such as high cytotoxic immune cell activity against cancer cells, pro-tumorogenic functions and increasing the expression of anti-apoptotic genes (15, 28). In a study by Zaharieva et al. (15), ellagitanines slightly but significantly inhibited the activation of NF-kB in human bladder cancer cell line (T24) than urinary bladder transitional cancer cell line (BC-3C). In addition, it was reported strong caspase 3 activation that cell proliferation decreases due to the increase in apoptosis rate (15). Losso et al. (29) found that EA applied to cell cultures at a concentration of 1-100 μmol/L had anti-proliferative effects against Caco-2 (human colon epidermal carcinoma cell line), MCF-7 (human breast cancer cell

line), Hs 578T (human breast cancer cell line) and DU 145 (prostate cancer cell line) cancer cells. However, with these concentrations, it was also reported that EA reduces the expression levels of vascular endothelial growth factor (VEGF)-165, matrix metalloproteinase (MMP)-2 and MMP-9 which are angiogenic factors in the tumor microenvironment (29).

In a study related with the radiosensitive effect of 10 µmol/L EA application on hepatocellular carcinoma cell growth was claimed an increase in caspase-3 activation and Bax proapoptotic protein level when Bcl-2 antiapoptotic protein level decreased (30). Thus, it was found that there was a shift in the Bax/Bcl-2 balance towards induction of apoptosis by EA application. It was also stated that oxidative stress which is characterized by increased levels of thiobarbituric acid reactive substances as a marker of lipid peroxidation and decreased levels of reduced glutathione as one of the endogenous antioxidants and loss of mitochondrial membrane potential and consequently the intrinsic apoptosis pathway can be manipulated by EA (30). It was claimed that administration of 40 and 80 mg/kg EA by oral gavage for 22 days alone in mice suppressed the increased expression levels of cancer-associated protein phosphatase 2A in mice with lung tumors and indicated anti-lung cancer activity for significantly inhibited tumor growth with increased autophagy (3, 31). In a study investigating the effect of EA on human cervical cancer cells, it was revealed that the groups treated with EA at 2.5, 5.0 and 10.0 µM concentrations reduced the invasion rate of HeLa cells to 76.43%, 65.54% and 56.44%, respectively (32).

3.   Conclusion

Considering various *in vivo* and *in vitro* experimental studies involving EA application, it is seen that anticarcinogenic effect of EA could cause significantly changes in intracellular and extracellular molecular analyzes. It is understood that EA has been keeping up-to-date in terms of interest in molecular therapy approaches for cancer since its discovery. In addition, the number of molecular medicine studies on EA and cancer continues to increase day by day with more complex studies.

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VITAMIN K AND
ITS PLACE IN LIFE

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1.   Introduction

Vitamin K (VK) alludes to a group of fat-soluble vitamins. VK is a compound serving as coenzyme of γ-glutamyl carboxylase, which catalyzes the carboxylation of glutamic acid (Glu) residues to γ-carboxyglutamic acid (Gla) residues. In this way, the vitamin K-dependent proteins (VKDPs) become active and the binding of calcium to the protein is ensured. These proteins are also called Gla-proteins. VK is necessary for normal coagulation since Gla-proteins in the blood clotting are dependent on VK. Gla proteins are not only involved in coagulation but are also associated with cardiovascular and bone mineralisation, diabetes, immune response, and cancer (1).

Natural forms of (VK) are phylloquinone (PK; vitamin K1) and menaquinones (MK; vitamin K2) (2). Because PK acts as an electron carrier in Photosystem I, it is produced by photosynthetic organisms such as cyanobacteria, algae, and green plants (3), while menaquinones are mostly produced by archaea, bacteria, and animals (1). Menadione (MD), often referred to as vitamin K3, is not found naturally in foods, but is a catabolic product of PK and a precursor to

circulating MK-4 (4). Currently, new synthetic forms of vitamin K have been identified. These are vitamin K4 (menadiol sodium phosphate) and vitamin K5 (4-amino-2-methyl-1-naphthol), the water-soluble form obtained by reduction from menadione. While VK1 is mainly important for blood coagulation, VK2 has been discovered to have a function in events such as cell proliferation, vascular calcification and bone metabolism. Anticarcinogenic effects of vitamins K2, K3, K4 and K5 have also been reported in some studies (5).

2. Structure of Vitamin K

The core structure of VK is a 2-methyl-1,4-naphthoquinone ring structure. The length and saturation of the isoprenyl side chain at position 3 of the naphthoquinone ring determine the type of VK (Figure 1). The PK is the only compound with 4 isoprenoid residues (one of the isoprenoid residues is unsaturated) in its aliphatic side chain. All of the isoprenoid residues in the side chain of MKs are unsaturated. The number of these isoprenoid residues is denoted by "n" (MK-n) and determines the length of the side chain (6). MKs are divided into short-chain (MK-4) and long-chain (MK-5 to -13) subtypes due to the variable number of isoprenoid units (4).
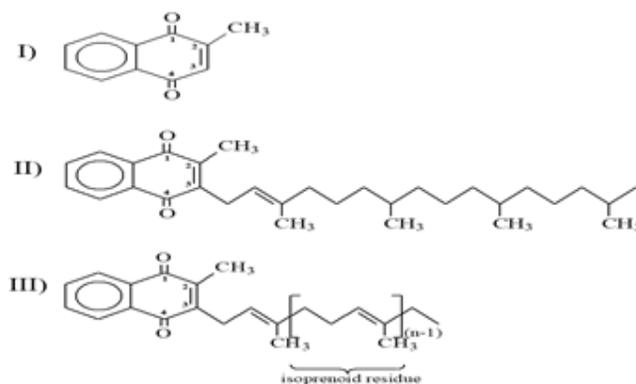
Figure 1: Structure of Vitamin K Forms, **I)** 2-methyl-1,4-naphthoquinone (Menadione; Vitamin K3), **II)** Phylloquinone (Vitamin K1), **III)** Menaquinones-n (Vitamin K2)

In recent studies, it has been discovered that MKs can be synthesized from other forms of VK by animals and humans. It has been determined that MK-4 is produced by bacteria in the gut by transformation from exogenous naphthoquinones (3). MD has the simplest structure and does not contain an aliphatic R group. Unlike

naturally occurring forms, MD is hydrophilic and not taken through diet (7). However, despite lacking biological activity, it acts as an intermediate in human metabolism (3) and is a provitamin for MK-4 to be synthesized in tissue (8). While the half-life time of MKs with longer side chains is around 72 hours, the half-life time of PK and MK-4 is about 1.5-2 hours (5). PK and MKs are catabolized in a common pathway in the liver. The polyisoprenoid side chains are shortened first. After ω-oxidation and β-oxidation, respectively, they are converted into two aglycones with side chains of five and seven carbon atoms (Figure 2). The glucuronides formed by the conjugation of these aglycones with glucuronic acid are excreted in the urine and bile (9).
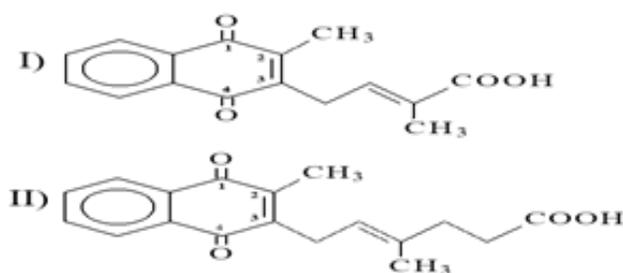
Figure 2: Vitamin K Metabolites. **I)** Aglycones with Side Chains of Five Carbon Atom, **II)** Aglycones with Side Chains of Seven Carbon Atom

3.   Sources of Vitamin K

Phylloquinone accounts for 90% of the vitamin K content of the human diet (2). Data on PK content can vary considerably as it can be influenced by several factors including species, growing method, growing location, climatic conditions, plant maturity, and method of determination (3). PK is only found in organisms capable of photosynthesis, including cyanobacteria, algae, and green plants (10-12). Because the green parts of plants contain high levels of PK, it was previously thought that PK exists only in chloroplasts. Subsequent studies have reported that it is found in peroxisomes and cell membranes, as well as in some non-photosynthetic parasitic plants (10,13,14). While green cruciferous vegetables (broccoli, brussels sprouts, etc.) are rich sources of phylloquinone, vegetables such as spinach, chard, and parsley also have significant phylloquinone content (6,15-17). In general, because the green parts of plants contain high amounts of phylloquinone, wild edible plants such as nettle, wild garlic, dandelion leaves, and elderberry are also included (18).

Some vegetable oils are one of the important dietary sources of the PK for humans. The most important of these oils in terms of PK content are soybean oil, rapeseed oil and olive oil (about 185, 130, and 55 µg/100 g, respectively) (6,19,20). PK in vegetable oils is relatively heat stable. Even after 40 minutes of heating at 185-190°C, there is a maximum 15% reduction in PK content. Therefore, cooking vegetables using vegetable oils can enrich the food with an extra dose of PK. Phylloquinone is extremely sensitive to sunlight and fluorescent light. The vegetable oil was exposed to these light sources for 2 days and it was observed that the PK content of the oil decreased by 46% and 87%, respectively. For this reason, it is recommended to store oils in a dark environment (20).

Although MKs can be produced in the human intestinal microflora, their production amount is quite low (2). Dairy products are among the main sources of MK in the human diet. Cheeses are very rich in vitamin K2 since they undergo bacterial fermentation during their production. About half of the menaquinones ingested by humans originate from cheese (21,22). Although menaquinone levels in dairy products differ according to the type of starter bacteria used, the dominant form is MK-9 (23,24). Fermented vegetables such as sauerkraut and natto (fermented soybean) are other important sources of MKs (25).

Although the intake of MK-4 with food is not high, it is stated that it is commonly found in some tissues (2). Since MD is an artificial form of VK, it is often added to fortified animal foods subsequently. Therefore, it must be converted to MK-4 in the liver to become active (26). In addition, other tissues (particularly the pancreas, vessel wall, brain, and testis) can convert phylloquinone to MK-4 (27,28). For these reasons, MK-4 levels in animal products (meat, dairy products, etc.) are relatively high (6). Menaquinones from MK-7 to MK-10 can be synthesized by intestinal bacteria as well as being found in fermented foods. Tissue MK-4 is derived primarily by an endogenous conversion from dietary phylloquinone, independently of the gut microbiota. The intestine can also break down dietary PK into MD and release it into the circulation (29,30).

4.   Absorption of Vitamin K

Intestinal absorption of VK is thought to follow the same pathway as other lipophilic compounds. In this pathway, VK is first taken up into mixed micelles, and these micelles are absorbed by enterocytes. They are packaged

as chylomicrons within enterocytes and released into the lymphatic system by exocytosis (1,31).

It is stated that the bioavailability of VK varies according to the intake of other nutrients in the diet, the structure of the nutrient matrix, pancreatic enzymes, and bile secretion (2). Due to the low-fat content of vegetables, PK has a lower bioavailability than other forms of VK (menaquinones)(32). While the absorption of PK is 5-10% when vegetables are not consumed with oil, the absorption rate increases to 10-15% when consumed with oil (2). In some sources, it has been reported that the amount of PK absorbed from plant products in humans varies between 4 and 64%, and it usually triples when cooked leafy vegetables are taken with oil (33,34).

5.   Distribution of Vitamin K

In terms of bioavailability and biodistribution, PK has a shorter half-life than that of MKs. While PK is preserved and functioning in the liver, MKs are redistributed into the circulation and extrahepatic tissues (4). Menaquinone-4 is the primary form of VK in the brain and some other extrahepatic tissues (35). MK-4 in tissues is produced primarily from dietary PK, independently of bacteria in the gut (29).

Since it is a fat-soluble vitamin, VK is absorbed from the intestinal lumen in the entity of bile salts and the pancreatic lipase, and then accumulates in the liver, spleen, and lungs, but cannot be stored in the body for a long time (7). VK has the lowest serum level among the fat-soluble vitamins in humans. Therefore, its metabolic recycling helps maintain adequate sources of VK(36). In mouse studies using a stable isotope, it has been observed that administration of PK and different MKs, separately and in combination, had an equivalent conversion to MK-4 in extrahepatic tissues (37).

6.   Physiological Functions of Vitamin K

Vitamin K is not generally used for clinical purposes. It is only given to newborns to prevent vitamin K deficiency bleeding. Both PK and MKs are essential for many VKDPs, such as coagulation factors (Factor II (prothrombin), VII, IX, X, Protein C, S, and Z), extrahepatic Gla protein (osteocalcin) in bones, and matrix Gla protein (MGP) in the vessel wall structure (9,38). The carboxylation reaction plays a critical role in binding calcium to VKPDs (2). High levels of VK are required for γ-carboxylation of osteocalcin (39). If osteocalcin can not

be carboxylated, it cannot bind to hydroxyapatite, so uncarboxylated osteocalcin levels in serum provide an idea of the metabolic cycle of bone (40).

It is known that VK has a role in performing the functions of several proteins that are involved in various processes (tissue mineralization, energy metabolism, inflammation, cellular growth) apart from blood coagulation (9,41). Currently, the use of VK in the prophylactic and clinical treatment of age-related chronic diseases (osteoporosis, and osteoarthritis), cardiovascular diseases, inflammatory diseases, neurological disorders and cancer has been discussed (4).

The presence of VKDPs has also been demonstrated in brain tissue and it has been notified that VK has an important role in cognitive health by mediating cognitive functions (42,43). Different studies have reported that higher VK intake improves cognitive performance or reduces the risk of Alzheimer's disease (44-46). In another study, it said that cognitively healthy elderly individuals with higher PK levels were better at cognitive assessments related to consolidation processes (47).

7. Vitamin K Deficiency

Vitamin K deficiency has not been reported in adults without pathological disorders. Because vitamin K is reused through the oxidation and reduction cycle and is synthesized by bacteria in the gut. It is given prophylactically only to prevent vitamin K deficiency bleeding (VKDB) in newborns (8).

In case of vitamin K deficiency. The European Food Safety Authority (EFSA) has set an adequate intake of 1 μg PK per kg of body weight for both sexes and all age groups. However, a higher amount of 90 μg/day for women and 120 μg/day for men has been established in the USA (48). In Italy (by the Italian Society for Human Nutrition) it has been reported that adequate VK intake should be 140 μg/day for people 18-59 years old and 170 μg/day for people over 60 years old. Adequate VK intake has not been definitively established in the UK, based on previous data it has been calculated that adequate intake should be 1 μg/kg body weight. This dose might be sufficient for normal blood clotting, but insufficient for other processes such as vascular calcification and bone metabolism (49). In the Czech Republic, the recommended daily intake for VK is 75 μg. (5).

It has been shown that excessive vitamin E intake causes VKDB (50), and excessive α-tocopherol intake prolongs active partial thromboplastin time (APTT) and prothrombin time (PT), which are markers of blood coagulation

(51-53). It has been demonstrated that the intake of α-tocopherol reduces the concentration of PK in extrahepatic tissues (54).

Higher VK intake reduces the risk of type 2 diabetes (22,55). In intervention studies, although the results are inconsistent (56), increased insulin sensitivity has been demonstrated after PK supplementation (57-59). It is not known exactly how insulin sensitivity is increased, but the possible the anti-inflammatory effect of vitamin K and carboxylation of osteocalcin have been recommended by researchers (59-61).

8.   Vitamin K and Covid 19

Coronavirus disease (COVID-19) is a respiratory tract infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although most people with this disease show mild symptoms, respiratory failure due to pneumonia, as well as symptoms such as coagulopathy and venous thromboembolism, have been observed (62,63). The cofactor of anticoagulant protein S is vitamin K, and most of it is synthesized extrahepaticly in endothelial cells (64). Carboxylation of extrahepatic vitamin K-dependent proteins is more severely affected by vitamin K deficiency. This suggests that low VK levels may cause increased thrombosis (65).

MGP also acts as an inhibitör of calcium deposition in arterial walls (66). VK supplementation resulted in a 50% reduction in atherosclerotic calcification because of the activation of more MGPs (67). Elastic fibers with high calcium affinity are the main matrix components of the lungs. There is a relationship between raised calcium deposition in elastic fibers and increased synthesis of matrix metalloproteinase (MMP). An increase in a subset of MMP-producing macrophages has been demonstrated in severe SARS-CoV-2 pneumonia (65).

Desphospho-carboxylated (dp-uc) MGP (inactive MGP) is a indicator of extrahepatic vitamin K status. In humans, elevated dp-ucMGP concentrations sign low extrahepatic vitamin K status and vice versa (36). The high level of dp-ucMGP in hospitalized patients due to COVID-19 indirectly brought to mind extrahepatic VK deficiency (65).

In cases of VK deficiency (intestinal malabsorption or during drug administration such as anticoagulants), an increase in inflammatory cytokine levels, including C-reactive protein and IL-6, has been observed (68). High IL-6 levels have been observed in COVID-19 patients admitted to intensive care units (69,70). In addition to its anti-inflammatory effect, high VK supplementation

has been shown to decrease the risk of cardiovascular disease and coronary calcification (71).

9. Determining Vitamin K Status

Of all the fat-soluble vitamins, vitamin K is the most lipophilic and least abundant, making it difficult to develop assays for the latest generation chemistry platforms (49). Indirect measurement methods such as prothrombin time or uncarboxylated osteocalcin and MGP (72) or direct measurement methods such as high-performance liquid chromatography (HPLC) (73) and liquid chromatography tandem mass spectrometry (LC-APCI-MS/MS) (74) are used to measure vitamin K status.

Direct measurement of blood VK levels is not convenient to assess VK levels because of differences in half-life and bioavailability between PKs and MKs, and also the fact that the uptake of MKs is too low to be measured accurately. Therefore, measuring circulating inactive VKDP levels is a more valuable method (75,76). dp-ucMGP is one of the indirect markers used to indicate non-hepatic VK status (76). Des-gamma-carboxy prothrombin, or the prothrombin induced by vitamin K absence II (PIVKA II) is an aberrant protein produced in the liver due to vitamin K deficiency. It has been reported that PIVKA II levels can be used as an indicator of VK deficiency (77).

Various methods have been developed based on the detection of the conversion of the quinone group in the content of VK to quinol as fluorescent or electrochemiluminescent. VK homologs can be directly determined with precision using different (post-column reduction procedures and fluorometric or electrochemical detection) HPLC (High-pressure liquid chromatography) methods. However, these methods require extensive sample purification before measurement because of the interference of lipids. The most current methods are tandem mass spectrometry (LC-MS/MS), which is based on the basic liquid chromatography method (49).

10. Conclusion

Since its discovery as a coagulation factor in 1935, it has been noticed that vitamin K is also essential for proteins responsible for various physiological processes, such as tissue mineralization, bone development, inflammation, and neuroprotection. Scientific studies have shown that an increase in vitamin K intake can reduce the risk of bone fractures, arterial calcification, inflammatory

diseases and cognitive decline. When all these physiological processes are considered together, it can be realized that vitamin K has an important place in human life.

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NEUROPROTECTIVE AND ANTICANCER BIOACTIVITY OF APIGENIN

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1. Introduction

The name "apigenin" comes from Apium due to its derivation from the Apium genus in the Apiaceae family (1). Apigenin (4′,5,7-trihydroxyflavone, $C_{15}H_{10}O_5$, 270.24 g/mol) (Figure 1) is a natural small-molecule biocompound commonly distributed in the plants, including apple, basil, celery, chamomile, grapefruit, chamomile, kumquats, grapes, onions, oranges, oregano, parsley, pepper, thyme (2,3). It's available an apigenin-7-O-glucoside (Figure 2) and/or acylated derivative from natural sources like chamomile (4). Moreover, it presents as a dimer structure, biapigenin (Figure 3), essentially separated from the bud and flower of *Hypericum perforatum* (5). In the pure form of apigenin, it exists as a yellow-coloured needles. Apigenin possesses strong solubility in dilute potassium hydroxide and dimethyl sulfoxide and mild solubility in hot alcohol. Apigenin must be kept at -20° C or lower temperature because it is not chemically stable at room temperature (6,7).

In the literature, apigenin was firstly studied by Spicak and Subrt (8) who determined its influence on the release of histamine in 1958. Zhang and colleagues (2) reported that apigenin could be absorbed in the whole intestine including ileum, colon, duodenum and jejunum segments, and also the essential absorption site was at duodenum. Moreover, it is known that apigenin crosses the blood-brain barrier (9). Because of its poor solubility and high permeability, Zhang and colleagues classified apigenin as a Biopharmaceutical Classification System Class

II medicine (2). Some studies have revealed that apigenin has anti-estrogenic activity (10,11). A wide range of *ex vivo* and *in vivo* researches throughout the years have presented that apigenin possesses various bioactivities such as anti-inflammatory (12), anti-oxidant (13), antiapoptotic (14), antigenotoxic (15), antiviral (16) anticancer (17,18), antiangiogenic (19), and neuroprotective (20). Moreover, in the literature, the potential protective influences of apigenin on cardiovascular and metabolic diseases were significantly emphasized due to its anti-inflammatory, antioxidant, and antiapoptotic activities, with a variety of *in vivo* and *ex vivo* research (14).

Nowadays, the most common pathologies which influence millions of humans all over the world are neurodegenerative diseases and cancers. Moreover, both are statistically acknowledged as the main reasons for morbidity and mortality around the world. Thus, the purpose of this chapter is to offer an update on apigenin's anticancer and neuroprotective bioactivity based on the findings of a variety of studies.

2. Neuroprotective bioactivity of apigenin

Apigenin is now widely recognized as a neuroprotective agent. Apigenin's high anti-inflammatory and antioxidant action have sparked interest in its potential neuroprotective properties. Because excessive creation of reactive oxygen species (ROS) is thought to be a trigger for many neurodegenerative diseases, there has been a surge in interest in the role of oxidative stress in neurodegeneration over the last two decades (21,22).

In recent studies, apigenin has been indicated to own strong neuroprotective activity in a variety of experimental animal models. Apigenin administration demonstrated neuroprotective effects on focal cerebral ischemia-reperfusion damage-induced rats (23). In a mouse model of localized ischemia caused by cerebral artery closure, apigenin supplementation prevented neuronal cell death (24). Chronic apigenin administration has been presented to change microglial morphology in the hippocampus in glial fibrillary acidic protein interleukin (IL) 6 transgenic mice and suppress ionized calcium-binding adapter molecule 1+ microglial activation (25). Apigenin protects hippocampus neuron cells from apoptosis caused by endoplasmic reticulum stress (26). Zhao et al. (27) reported that apigenin has a positive role in cognitive function in Alzheimer's illness. In APP / PS1 double transgenic Alzheimer's disease experimental model, they found that apigenin caused memory retention and learning deficits when compared to the Alzheimer's disease group.

The molecular mechanism of the neuroprotective influence of apigenin has been tried to be revealed by many researchers. Apigenin exerted its neuroprotective effects by activating the signaling pathway of phosphoinositide 3 kinase (PI3K) / Akt / nuclear factor erythroid 2 related factor 2 in hypoxic-ischemic brain damage in the neonatal rat (19). Apigenin supplementation has been indicated to augment learning and memory abilities. Maintaining neurovascular functions and reducing oxidative damage dysregulation of brain-derived neurotrophic factor (BDNF), tropomyosin receptor kinase B, and phospho cyclic adenosine monophosphate response element-binding protein (CREB) levels in amyloid-beta (Aβ) 25-35-induced amnesic rats (28).

In an experimental Alzheimer's disease model induced by copper-mediated beta-amyloid neurotoxic action, apigenin exerted a neuroprotective effect by maintaining the mitochondrial function, suppressing neuronal apoptosis, and suppressing the signaling pathway of mitogen-activated protein kinase (MAPK) in cell culture (29). Apigenin therapy (a 90-day treatment at 40 mg/kg) enhanced memory retention and learning impairments in an experimental Alzheimer's disease model with APP / PS1 double transgenic mice. Moreover, apigenin reduced fibrillar amyloid deposition which was determined by the Thioflavin S staining test. Apigenin therapy also lowered the activity of β-site amyloid precursor protein-cleaving enzyme 1 and the concentrations of insoluble $A\beta_{1-40}$ / $A\beta_{1-42}$. It inhibited *β-Amyloidogenesis* process, as well as elevated the values of BDNF and CREB in the cerebral cortex (27). In another investigation, Liu and colleagues (28) found that apigenin therapy (20 mg/kg for eight days) improved the cholinergic system and microvascular function in the Aβ 25-35 induced mice model of amnesia. The mechanism of apigenin on the cholinergic system was explained by the supression of acetylcholinesterase activity. The signaling pathway of extracellular signal-regulated protein kinase (ERK) / CREB / BDNF has been revealed to be dysregulated in Alzheimer's illness (30,31) and can be restored with apigenin treatment (27,32). In murine hippocampal neuronal (HT22 cell line) cells, according to Choi et al. (26), apigenin possesses antiapoptotic properties towards apoptosis that are triggered by endoplasmic reticulum stress. Furthermore, Balez et al. (33) found that apigenin has neuroprotective properties against apoptosis, the excitability of neuronal, and inflammation in a human induced pluripotent stem cell (iPSC cell line) model of sporadic and familial Alzheimer's disease. Taken together all this evidence, it appears that apigenin's potential protection against Alzheimer's disease progression involves multiple mechanisms.

Apigenin can have antidepressant-like effects as reported by some authors. In mice forced to swim test, apigenin was reported to increase the declined dopamine turnover in the amygdala (34). Monoamine oxidase (MAO) possesses an important role in removing neurotransmitters including dopamine, serotonin, and norepinephrine in the brain. Basically, there are 2 types of MAO (MAO-B and MAO-A). The inhibitors of selective MAO-A are used for depression treatment via elevating the concentrations of noradrenaline and serotonin. Despite this, a specific MAO-B inhibitor is used to treat Parkinson's disease symptoms (35). Han and colleagues (36) investigated the influences of apigenin on MAO and they presented that apigenin blocked both MAO-B and MAO-A levels. Chaurasiya et al. (37) have found that apigenin extracted from propolis selectively inhibits MAO-A rather than MAO-B. Apigenin improved impairments in central monoaminergic neurotransmitter and the systems of adenylyl cyclase activity in chronic mild stress depressed rats (38). Apigenin reduced oxidative stress, blocked NOD-like receptor family pyrin domain-containing 3 activation, and decreased the amounts of IL 1β and IL 18 In chronic unpredictable mild stress rats (39). In a research (20) related to the antidepressive features of apigenin in depressed animals that were subjected to both splash and forced swimming behavioral test, it has been presented new information that its antidepressant effects could be associated with the reduction of malondialdehyde level and the enhancement cellular antioxidants including glutathione, fluorescence recovery after photobleaching and coenzyme Q10 level and recovered inflammatory signaling pathways.

It has been reported that apigenin exhibits neuroprotection in an experimental Parkinson's disease model induced by rotenone. It was found that apigenin prevented a decrease of BDNF and glial cell line derived neurotrophic factor and an increase of nuclear factor kappa B (NF-κB). Apigenin also diminished the levels of IL 6, inducible nitric oxide synthase-1, and tumor necrosis factor alpha (TNF-α) while increasing dopamine D2 receptors expression (40). In another study, in a Parkinson's model induced with rotenone in rats, rotenone-induced symptoms (such as impaired motor coordination, postural instability, and decrease in rearing behavior) were ameliorated during a 14-day apigenin therapy at 10 mg/kg and 20 mg/kg (41). The present data suggest that apigenin could be used as a Parkinson's disease treatment drug.

3. Anticancer bioactivity of apigenin

It wasn't known until the 1980 years that apigenin, a non-mutagenic and low-toxic bioflavonoid, was related to the carcinogenesis process when Birt and coworkers (42) stated the effective antipromotion and antimutagenic features of apigenin. Apigenin has been shown to be effective as an antitumoral agent for a wide range of human cancers, such as breast, skin, bone, esophageal, pancreatic, liver, lung, bladder, prostate, colon, ovarian, and cervical cancers, in both *ex vivo* and *in vivo* studies (18,43,44).

Birt and colleagues (45) showed that apigenin application to SHK-1 mice resulted in reduced the activity of ornithine decarboxylase and a decline in size and number of skin carcinogenesis induced by ultraviolet-B light irradiation. Meanwhile, apigenin has been known as a keen inhibitor of ornithine decarboxylase that acts an essential role in skin cancer promotion (45,46). Apigenin inhibits 12-O-tetradecanoyl-phorbol-13-acetate-mediated cancer promotion in mouse skin, according to Huang et al. (47) by blocking protein kinase C. Wei et al. (46) demonstrated that topically apigenin application inhibits skin papillomas and indicates the tendency to diminish the conversion of papillomas to cancers by dimethyl benzanthracene-induced in mice. According to Lin and coworkers, apigenin inhibited osteosarcoma xenograft tumor development in vivo (nude mice bearing U-2 OS xenograft cancers) and triggered apoptosis via mitochondrial malfunction ex vivo (U-2 OS human osteosarcoma cell line) (48).

Apigenin suppresses the invasion of tumor cell via Matrigel, cell proliferation, and migration in an estrogen-insensitive breast carcinoma (MDA-MB231 cell line) cells, according to Lindenmeyer and colleagues (49). Apigenin has been shown in prostate cancer (DU-145 cell line) cells and breast cancer (MDA-MB-231 cell culture) cells to inhibit cancer cell growth via estrogen receptor beta (11). Way and coworkers (50) revealed that apigenin suppressed the development of human epidermal growth factor receptor (HER) 2 / neu-overexpressing breast cancer cells by inhibiting HER2 / HER3-PI3K / Akt signaling pathway inducing the apoptosis. Furthermore, apigenin has been found to induce apoptosis in human breast cancer (MDA-MB-453 cell line) cells by participating in all apoptotic pathways (51). Apigenin was indicated to possess killing influences on human esophageal cancer (KYSE150 and EC9706 cell lines) cells by blocking cell growth and proliferation, inducing apoptosis and with induction of the differentnesses in cell membrane due to toxicity (52). Apigenin has been demonstrated to induce apoptosis and DNA damage,

up-regulate the Bax, cytochrome c, caspase 3, apoptosis-inducing factor, GRP78 and GADD153, down-regulate Bid, B cell lymphoma 2 (Bcl 2), procaspase 8, reduce mitochondrial membrane potential and increase calcium ions (Ca^{+2}) and ROS in human lung cancer (H460 cell culture) cells (53).

Additionally, apigenin has been indicated to possess anticancer activities by inducing apoptosis mechanism via nicotinamide adenine dinucleotide phosphate oxidase activation in human hepatoma (HepG2 cell line) cells (54). Apigenin's powerful pro-apoptotic and antiproliferative activities *ex vivo* have been shown to block the growth of pancreatic cancer cells by causing cell cycle arrest (55). The relationship between the antineoplastic mechanism of apigenin and p53 function in pancreatic cancer (MiaPaCa 2 and BxPC 3 cell line) cells has been indicated by King et al. (56).

Furthermore, apigenin has been presented to block cell invasion and cell migration as well as suppress proliferation, in bladder cancer (T24 cell line) cells, by activating the signaling pathway of PI3K / Akt and the proteins of the Bcl 2 family, as well as inducing cell cycle arrest and apoptosis in a caspase-dependent way by increasing especially caspase-3 (57). Apigenin, according to Shi and coworkers (58), suppresses the growth of human bladder cancer cells (T-24 cell line) by inducing apoptosis and suppressing the progression of the cell cycle.

Dross and colleagues (59) indicated apigenin modulates the MAPK cascade in two different epithelial cell lines including HCT116 colon carcinoma cells and 308 mouse keratinocytes by p38 kinase and ERK, but possesses little influence on c-Jun amino-terminal kinase (JNK) phosphorylation. Lee and coworkers (60) showed that apigenin possesses the inducing effects of autophagy and apoptosis in human colon carcinoma (HCT116 cell line) cells. Apigenin suppresses the development of human colon cancer (HCT116 cell culture) cells, arrests the cell cycle, elevates Ca^{+2} and ROS levels, and inhibits matrix metallopeptidase (MMP) activity, according to Wang and Zhao (61).

Gupta and colleagues (62) presented that apigenin blocks the development of androgen-responsive human prostate carcinoma (LNCaP cell line) cells by reducing in the expression of androgen receptor protein along with a reduction in intracellular and released prostate-specific antigen forms. According to Shukla and Gupta (63), apigenin possesses antiproliferative and antitumoral properties in human prostate cancer cells, due to modulations in PI3K-Akt and MAPK, as well as deprivation of cyclin D1 related retinoblastoma dephosphorylation. Kaur and coworkers (64) have shown that apigenin led to induced the activity

caspase 9 and diminished survival cancer cells by inactivating Akt to trigger apoptosis in human prostate cancer (PC 3 cell line) cells. Moreover, in the same study, they indicated that oral apigenin intake resulted in Akt inactivation and apoptosis induction in prostate cancer cells 3 tumors *in vivo*. Shukla et al. (65) revealed that androgen-refractory human prostate cancer (DU145 and PC-3 cell line) cells with the treatment of apigenin led to a important decrease in the viability of cell and apoptosis induction with the increase of cytochrome C in a time-dependent way and in dose-dependent manner suppression of survivin and inhibitor of apoptosis (IAP) family of proteins such as c-IAP1, c-IAP2, XIAP values, meanwhile increasing in the active form of Bax protein and decreasing in Bcl 2 and B cell lymphoma extra-large (Bcl-xL) were accompanied by the mentioned influences of apigenin. Gupta et al. (66) presented that apigenin can influence the steady-state cell population by inhibiting the selective growth, responding to apoptosis, and deregulating the cell-cycle in a normal versus human prostate carcinoma cells.

Fang and colleagues (67) revealed that apigenin inhibits vascular endothelial growth factor expression, which is needed for angiogenesis and tumor growth, as well as hypoxia-inducible factor 1 alpha in human ovarian cancer cells. According to Li and coworkers (68), apigenin decreased inhibitor of differentiation or DNA binding protein 1 expression via activating transcription factor 3 and prevented carcinogenesis and proliferation in human ovarian cancer (A2780 cell line) cells. According to Tang and colleagues (69), apigenin restricted the self-renewal potential of human ovarian cancer (SKOV3 cell line) generated sphere-forming cells by downregulating the expression of glioma-related oncogene 1 with casein kinase 2 inhibition alpha. Apigenin lowered the levels of Tyro3 and Axl receptor tyrosine kinases, Bcl-xL, and Akt phosphorylation in ovarian cancer (SKOV3 and SKOV3 / TR cell line) cells, according to Suh et al. (70). Apigenin was suggested to be a possible anticancer agent alternative for ovarian cancer, which is known as the fifth essential reason of pre-senescent death in women, (71) as it was presented to block the survival and growth of ovarian adenocarcinoma cells (SKOV-3 cell line) in both manners a dose-dependent and time-dependent.

Furthermore, apigenin has been shown to reduce Bcl 2 protein expression and impede the proliferation of human cervical carcinoma (HeLa cell culture) cells via the p53-dependent signaling pathway (72). Souza et al. (73) presented that apigenin can block the invasion and migration of different cancer cells such as C33A, HeLa, CaSki, SiHa, and HaCaT cell line. Apigenin blocked the

formation of cervical cancer cells in mouse, according to Chen et al. (43) who found that it prevented cervical cancers. Moreover, it was indicated that the molecular signaling processes of apigenin in cervical cancer both *in vitro* (C33A cell line and HeLa cell line) and *in vivo* were related to its inhibiting effect on PI3K/Akt signaling pathway (PI3K, mammalian target of rapamycin, and Akt) and focal adhesion kinase (FAK) signaling pathway (FAK, integrin β1, and paxillin). Additionally, apigenin has been indicated to mediate antitumoral activities through molecular processes potentially including activation of caspase 8, caspase 3, TNF-α and Bax; inactivation of snail family transcriptional repressor (SNAI)1, SNAI2, MMP-2, Bcl-2, and MMP-9; reducing the expression of Akt, ERK, NF-κB, MAPK, phospho-Akt, PI3K, p38, and JNK; and activating the degradation of proteasomal Her2/neu protein (44). Apigenin has recently gained popularity as a nonmutagenic chemopreventive agent. Apigenin may be improved as a promising chemotherapeutic and/or chemopreventive drug against a variety of cancers, according to the literature review.

4.   Conclusion

According to the literature review, apigenin, a bioactive compound, has remarkable features. It has functional activities that represent it could have an essential role in the prevention of a wide range of illnesses linked to neurodegenerative disorders and a large number of cancer types. Until now, apigenin has been proposed as both an antitumor and a neuroprotective agent in numerous various researches. Thus, it is thought that apigenin can be developed as a promising neuroprotective and chemopreventive drug for humans. Moreover, several issues have to be further researched especially the reliance and effective doses of apigenin. In the meantime, further study is needed to elucidate the most precise mechanisms of apigenin's molecular signaling effects in each of the diseases.

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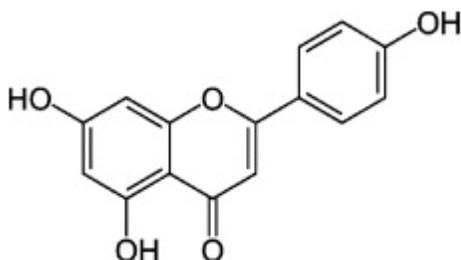
Figure 1. Chemical structure of apigenin
(5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-ben-zopyran-4-one) (2).
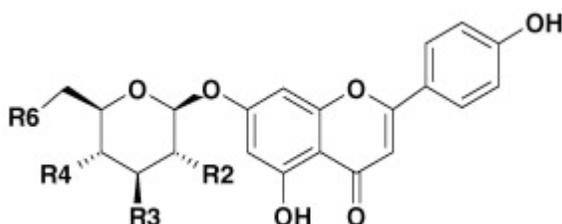
Figure 2. Structural formula of apigenin glucoside (4).
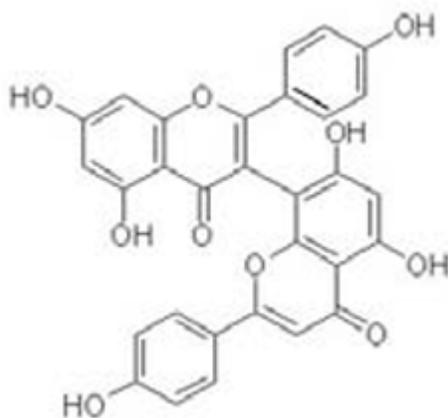
Figure 3. Structural formula of biapigenin (5).

SEVERAL ASPECTS OF CAFFEINE

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1.   Introduction

Caffeine (1,3,7-trimethylxanthine) is a widely used and commercially important plant-derived purine alkaloid. It is recognized as the most popular psychoactive drug worldwide (1) and is one of the most important components and natural stimulants in widely consumed beverages, including coffee, tea, soft drinks, energy drinks, and cocoa. This alkaloid is a nitrogenous organic compound known as "mateine guanine", which is among the substances that stimulate the brain, and contains 49.5% carbon (C), 5.2% hydrogen (H), 28.9% nitrogen (N), and 16.5% oxygen (O). Caffeine has a slightly bitter taste and is colorless and odorless; at room temperature, it is in powder form (2).

German scientist Friedrich Ferdinand Runge isolated caffeine from the coffee plant for the first time in the early 1820s (3), and it has since been isolated from the leaves of many plant species and used in a variety of applications in the food and cosmetic industries (2,3). This alkaloid is mostly synthesized during the growth phase of the plant, in its young fruits and leaves. The caffeine content in plants can vary depending on the plant species and different organs within the same plant; however, as the plants mature, the caffeine concentration is directly proportional to the plant's species, size, and growing conditions (3).

It is estimated that approximately 120.000 T caffein are annually consumed worldwide (1). Statistical data have revealed that in 2020 and 2021, global coffee consumption rose by as much as 166.63 million people, slightly increasing from previous years (4). Health problems and warnings are generally associated with excessive consumption of caffeine (3,5). It has been reported that 1.000 mg caffeine (about five cups of espresso) can lead to problems with conception, severe stomach aches, and intestinal disorders (5).

2. Synthesis

Caffeine is synthesized in a way similar to that of purine nucleotides produced by *de novo* biosynthesis or salvage pathways. The first step in the biosynthesis of caffeine and other methylxanthines is the conversion of adenosine monophosphate (AMP), inosine monophosphate (IMP), xanthine monophosphate (XMP), and guanosine monophosphate (GMP) into xanthosine. The four-step conversion of xanthosine into caffeine consists of three methylation steps and a nucleosidase reaction. The first step is xanthosine methylation N-methyltransferase attaching to S-adenosyl-L-methionine (6). Xanthosine is converted into 7-methylxanthosine by 7-methylxanthosine synthase. Next, 7-methylxanthosine is hydrolyzed by a nucleosidase to form 7-methylxanthine. Finally, caffeine synthase converts 7-methylxanthine into caffeine using theobromine (3).

Xanthosine, the first substrate in the synthesis of purine alkaloid, is synthesized in at least four different ways—the *de novo* purine biosynthesis (*de novo* pathway), adenine (AMP pathway) and guanine nucleotide (GMP pathway), and the S-adenosyl-L-methionine (SAM) cycle (SAM pathway) (6).
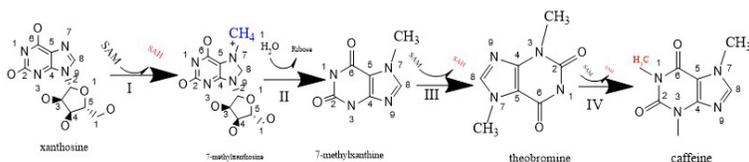
Figure 1. Main pathways of caffeine synthesis from xanthosine.

I)7-methylxanthosine synthase (xanthosine N-methyltransferase); II) N-methyl nucleosidase; III) theobromine synthase (monomethylxanthine N-methyltransferase); IV) caffeine synthase (dimethylxanthine N methyltransferase); III-IV)bifunctional caffeine synthase. Steps I and II are catalyzed by xanthosine N-methyltransferase (6).

Notes: SAM: S-adenosyl-L-methionine; SAH: S-adenosyl-L-homocysteine.

3. Degradation

Degradation of caffeine from coffee leaves was first reported by Kalberer (1965) (6). Caffeine is produced in actively growing parts of the plant, such as leaves, buds, and fruits, and is catabolized by two known pathways—N-demethylation and C-8 oxidation (3).

In most plants, N-demethylation is used for caffeine catabolism; however, it has been reported that the C-8 oxidation pathway is used in some plant species. In the N-demethylation pathway, caffeine is first demethylated at the N-7 position to form theophylline. This is the rate-limiting step of caffeine catabolism, which leads to the accumulation of caffeine in the plant's structure. Theophylline then undergoes two sequential demethylation reactions from the N-1 and N-3 positions to produce xanthine. Xanthine is then oxidized into uric acid and enters the purine catabolism pathway to produce allantoin and allantoic acid, which then breaks down into carbon dioxide (CO_2) and ammonia (NH_3). In some plant species, caffeine also undergoes direct oxidation from the C-8 position to produce 1,3,7-trimethyluric acid (TMU), after which it is methylated at the N-9 position to produce theacrine. Theacrine is then converted into liberin by methylliberine (3).

4. Digestion

Caffeine is absorbed by the stomach and small intestine within 45 min of consumption and then quickly spreads to the entire body; however, its

absorption can be affected by chemical and physical properties, pH, and the way by which it is administered. Because of its lipophilic property, caffeine crosses the blood–brain barrier and penetrates biological membranes. It has been reported that the half-life of caffeine is directly related to dose. Doses <10 mg have a half-life between 2.5 and 10 h, while higher doses prolong this half-life. It is metabolized by the liver cytochrome p450 oxidase system (CYP1A2) (1), and 75% is eliminated from the body within 6–7 h. Liver metabolism results in the production of paraxanthine, theophylline, and theobromine (3).

Almost all of the caffeine taken into the body is metabolized, but 3% or less is eliminated directly in the urin (7,8). The main pathway of caffeine metabolism in humans is the paraxanthine-n-3 demethylation pathway, known as 17X or 1.7 dimethylxanthine (7–9). This degradation takes place in the liver with the help of cytochrome p450 1A2 enzyme (8). It is estimated that theobromine, theophylline and 1,3,7-trimethyluric acid are formed as a result of studies with liver microsomes (10).

5.   Effects on Physiological Functions

5.1. Circadian rhythms

The circadian rhythm is the main regulator of the sleep–wake cycle as well as other physiological processes (11). Studies investigating the effect of caffeine on circadian rhythms have reported that it affects this cycle (11–13). As caffeine blocks adenosine receptors to delay sleep pressure, it alerts the central nervous system (CNS) (13). One study has reported that caffeine intake in the evening may delay the release of melatonin (11). It not only directly affects sleep but is widely used to resolve sleep-related problems (e.g., excessive daytime sleepiness) (13).
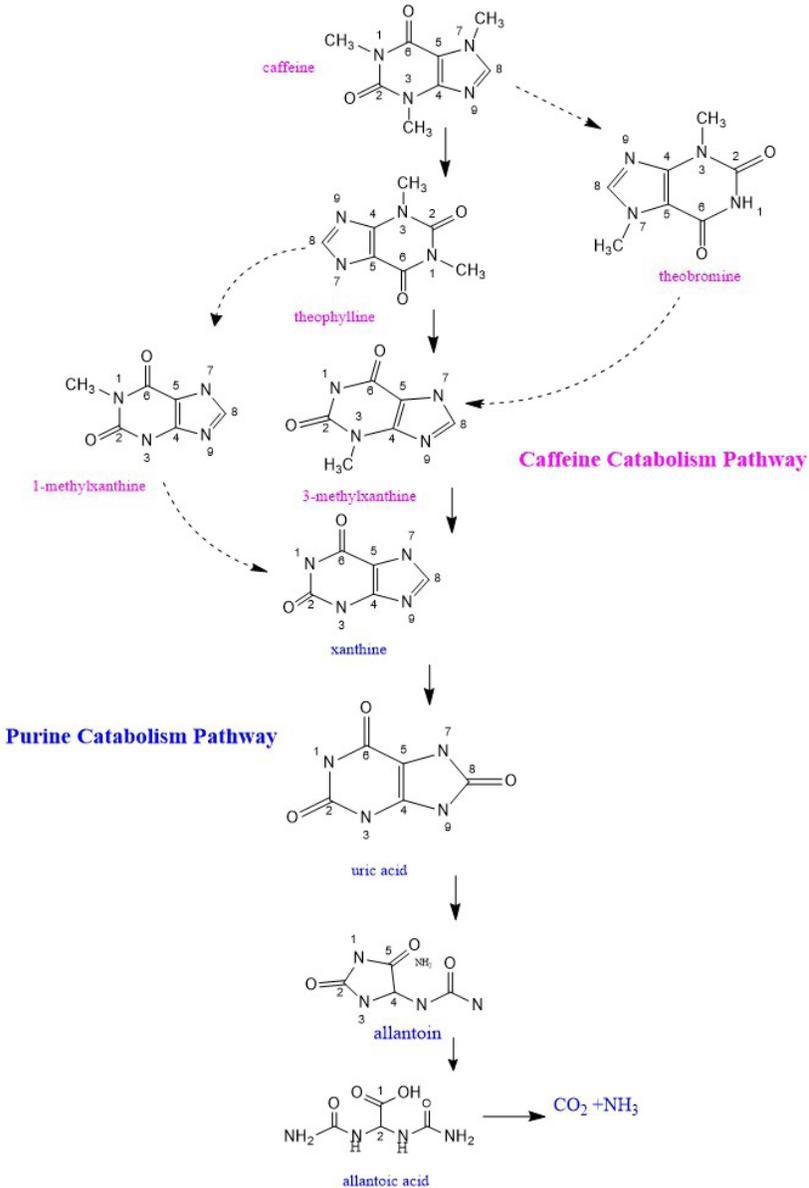
Figure 2. The catabolic pathways of caffeine.

Caffeine is mainly catabolized into xanthine by theophylline and 3-methylxanthine. Xanthine is reduced to carbon dioxide (CO_2) and ammonia (NH_3) through the oxidative purine catabolic pathway. Dotted arrows indicate secondary pathways. The conversion of caffeine into theophylline has been reported as a rate-limiting step (6).

5.2 Adenosine receptors

Caffeine has three important mechanisms of action that create a psychostimulant effect on CNS. Various studies involving humans and other animals have reported these effects (14).

One study has determined that the caffeine administered intraperitoneally into the body tissues accumulated day by day, with the highest increase being in brain tissue (15). One study found that running time on a treadmill was 60% longer in the group given caffeine (16).

The initial mechanism of action for caffeine is as an adenosine receptor antagonist for adenosine receptors (A)1, A2A, and A2B (14). Caffeine, as an A1/A2A receptor antagonist, and exhibits various stimulating effects on CNS, which leads to reduced drowsiness and increased activity within the musculoskeletal system (17).

Caffeine regulates adenosine receptors and the release of adenosine neurotransmitters in the brain and plays an important role in regulating sleep, arousal, consciousness, memory, and learning. By binding to adenosine receptors, caffeine blocks it from binding to its receptors. This blockage indirectly affects the release of neurotransmitters, such as norepinephrine, dopamine, acetylcholine, serotonin, glutamate, and gamma-aminobutyric acid (GABA). These neurotransmitters, in turn, affect mood, memory, alertness, and cognitive function (14).

5.3 Calcium

The effect of caffeine on the mobilization of intercellular calcium can be noted as a second mechanism of action. Caffeine helps calcium move across the sarcoplasmic reticulum and plasma membrane, where it is then released into the peripheral nervous system and CNS through synaptic transmission. At low concentrations of caffeine, calcium uptake through the endoplasmic reticulum is increased, which then decreases at higher concentrations; however, these effects are not related to caffeine's suppression of adenosine, GABA, or noradrenaline. In fact, there are three different intracellular calcium pools within the body. It has been reported that the second and third calcium pools are sensitive to calcium release that is induced by low doses of caffeine ingestion (14).

5.4 Fatty tissue and obesity

Caffeine's third mechanism of action is the inhibition of phosphodiesterases (14). Caffeine acts on the sympathetic nervous system and causes the secretion

of catecholamines, such as epinephrine and norepinephrine. This accelerates lipolysis by increasing the formation of cyclic AMP (cAMP) within the cell. Thus, caffeine acts as a regulator of fat metabolism. This phosphodiesterase inhibition causes cAMP accumulation and thus can increase lipolysis by breaking down triglycerides in adipose tissue into glycerol and free fatty acids. One study has found that the inhibitory effect of caffeine was highest in adipose tissue; therefore, it was suggested that caffeine can be used in the treatment of obesity (15).

One study on overweight and obese individuals in the United States has reported that caffeine consumption reduced the mortality rate of overweight or obese individuals by reducing oxidative stress; however, although caffeine intake reduced the risk of death in overweight people, this reduction was not observed in obese patients (18). Another study has found that caffeine consumption increased total serum cholesterol, lipoprotein a, and low-density lipoprotein (LDL) levels (19); however, another study has determined that in addition to high total serum cholesterol and LDL levels, the prevalence of coronary heart diseases and hypertension was also high (18).

5.5   Analgesia

Various effects of caffeine on pain have been reported (20). Caffeine is known to reduce the sensation of pain by inhibiting adenosine receptors (21). Together with its effect on A2A and A2B receptors, the inhibition of cyclooxygenase activity may also explain caffeine's analgesic effects as it enhances the effect of analgesic components. Recently, caffeine has been added to blends containing aspirin, acetaminophen, and other nonsteroidal anti-inflammatory drugs. Although caffeine was initially believed to only counterbalance the effects of analgesics, it is now considered to be an aid to other painkillers (20).Caffeine is also found in many over-the-counter medications, such as headache and appetite suppressants used with aspirin (22).

Some studies have reported that adding a small amount of caffeine to commonly used analgesics increased the effectiveness of pain relief in a small but significant portion of patients when compared with the use of analgesics alone (21,23).

5.6.   Oxidative stress

Some studies have shown that caffeine protects organisms from lipid peroxidation, the membranes from the effects of reactive oxygen species (ROS), and the liver from the harmful effects of radiation and other agents (24,25);

however, with regard to inhibiting the effects of ROS and peroxide radicals, some studies have found conflicting results (26,27). One such study has determined that high-dose intraperitoneal caffeine (100–150 mg) administered to rats after experimental head trauma increased lipid peroxidation and caused oxidative stress in the cerebral cortex (27). Another study has reported that administration of allyl alcohol and oral caffeine (150 mg) to rats with hepatotoxicity increased liver MDA levels (28). Caffeine destroyed hydroxyl radicals formed by the Fenton reaction ($Fe+2/H_2O_2$) (26). Another study has indicated that liver lipid peroxidation decreased and that cell membranes were protected from damage caused by ROS in rats given different doses of caffeine. The antioxidant activity of caffeine on liver metabolism supports the use of caffeine to treat oxidative stress (29).

It was found that consuming two cups of coffee a day reduced the toxicity caused by radiation in some cancer patients (30). One study has observed that adding caffeine (5–15 mg/kg) to the drinking water of mice rapidly decreased gamma ray–induced chromosomal damage (31).

To determine the effective dose of caffeine for this and to understand its mechanisms on the antioxidant system, the study has suggested that additional experimental studies be diversified (29).

6. Conclusion

Caffeine is a widely consumed plant-derived methylxanthine that is considered a CNS stimulant and has various effects on an organism. Among the mechanisms of action of caffeine, the focus has been on adenosine receptor antagonism and studies on this subject have continued. Because caffeine is an adenosine receptor antagonist, it accelerates lipolysis, especially in adipose tissue, and can be an agent to reduce the occurrence of obesity. The analgesic and antioxidant effects of caffeine are other important aspects of this plant-derived substance, although, it remains a controversial issue whether there are alternative solutions for reducing oxidative stress and pain within the body. We suggest that although caffeine has an important history of consumption worldwide, which continues to increase, more research should be conducted on its physiological effects.

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SOME ETHNOBOTANICAL PLANTS IN KARS (EASTERN ANATOLIA) AND THEIR ANTIMICROBIAL PROPERTIES

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1.   Introduction

Ethnobotany, a branch of ethnobiology, was first used by the American botanist John W. Harshberger in 1895 during his studies on plant use by indigenous peoples, using the term ethnobotany. The work titled "The Principles of eEhnobotany", published in 1896, is generally accepted as the beginning of ethnobotanical studies. (1). Ethnobotany is generally defined as the study of the relationship between plants and humans. (2). Throughout the history of humanity, mankind has benefited from the plants in its region for different purposes such as food and medicine. Previously, plants collected from nature were used, then these plants were cultivated and produced (3). Plants produce a number of chemicals known as secondary metabolites. Many secondary metabolites have been used by humans for various purposes, especially in drug making and as Homeopathy, Allopathy, Ayurvedic medicine (Doğu Anadolu). Many plant extracts and essential oils isolated from plants have been shown to exhibit biological activity in vitro and in vivo, which justifies traditional medicine research focused on the characterization of the antimicrobial activity of these plants (4). Asteraceae is one of the largest families in the Flora of

Turkey as well as in the world. It is also one of the most important families in Turkey where many popular plants are used as food and relaxation tea. (5). The general characteristics, distribution and antimicrobial properties of *Achillea arabica* Kotschy, *Achillea millefolium* L., *Tanacetum balsamita* L., *Artemisia absinthium* L. and *Cichorium intybus* L. species, which are widely used in Kars and are members of the asteraceaea family, were investigated.

2.   Some ethnobotanical plants in Kars (Eastern Anatolıa)

2.1. *Achillea arabica Kotschy*

(Synonyms: *Achillea biebersteinii* Hub.-Mor., *Achillea crithmifolia* Hampe)

The height of *A. arabica* Kotschy, whose Turkish name is "hanzabel", varies between 10-100 cm. The stem longitudinally striped and densely spreading-pubescent. The leaves are loosely or densly pilose. The inflorescence is 2-10 cm wide and consists of 30-200 capitula (Figure 1). It grows in coniferous forests, steppe, dry meadows, rocky slopes, and fallow fields. The altitudes it lives at are between 350-3450 m. Hanzebel's flowering period is between April and July. The phytogeographic region which the plant belongs is the Irano-Turanian phytogeographic region (6,7).


Figure 1. *A. arabica* Kotschy

The distribution of *Achillea arabica* Kotschy in Türkiye is given in Figure 2, and its distribution in the world is given in Figure 3.

Figure 2. Türkiye distribution map of *A. arabica* Kotschy (7)

■ Native. ■ Introduced

Figure 3. World distribution map of *A. arabica* Kotschy (8).

In the studies, it observed that the hanzabel plant has many local names as civan perçemi (9), sarı civanperçemi (10-12), pazvat, amel otu, ayvadana, yayla çiçeği, sarılık otu, arı çiçeği, (5), ormanderen (13), mayalık otu, sırçanotu, kurtotu (14), kulilkamera, ısfaysara, sıvüsra, gihayê mera (1), bovijan (15), oymadere, ormaderen, ormadere, kılıç otu (16), yılan pungu (17), hanzabel, pung, hayye, verdel (18), ayvadenesi, anababa (19), çiçegizer, melhem, pıltane zer, kem (20).

According to studies, fresh flowers of the *Achillea arabica* Kotschy plant are pounded and eaten raw to treat stomachache. A decoction of dried flowers is used to treat stomachache, as an antidiabetic and to lower cholesterol (11). Tea in the form of infusion prepared from its seeds is used in colds, back and rheumatic pains (12). The aerial parts of the plant are dried and boiled in water and drunk on an empty stomach for menstrual pain, menstrual irregularity, stomachache and nausea, uterine inflammation (9,10). In addition, for the treatment of sinusitis pain, the plant is boiled in water and the head area is kept in its steam (10). Tea prepared with infusion of flowers is used internally as a carminative, appetizing, energizing, diuretic, in colds, asthma, heart palpitations, kidney pain. The newly emerged leaves of the plant are crushed and applied externally to stop the blood in cuts. The leaves of the plant are crushed and mixed with the tail fat of the sheep. The prepared mixture is used externally as an ointment for wounds. After the leaves are boiled, they are crushed and applied as a mask on the skin spots on the face. Mosquitoes are prevented by placing the plant next to open food in the kitchen (16). Inflorescence decoction of the plant is used for cystic structures in gynecological diseases. For sty in the eye, the flowers of the plant are boiled and left to warm, then cotton is dipped in the water and rubbed into the eye. In diabetes, the aerial parts of the plant are boiled and the water is drunk. The decoction of the leaves and flowers of the plant is drunk as an anti-inflammatory. As a cough suppressant, the aerial parts of the plant are brewed like tea and the juice is drunk. In the treatment of wounds, the flowers of the plant are dried and powdered and applied to the injured area through a cheesecloth. In hemorrhoid disease, the leaves of the plant are applied as pulp (1). It can be boiled and drunk with water or milk. It is good for digestion, colds, liver inflammations. It is known that if women enter a tub of yarrow boiled with milk, it has the ability to heal all diseases in the uterus. Decocsion of *A. arabica* Kotschy prepared as a tea and it is used in the treatment of cough, cold forehead, jaundice, tonsil pain and tooth inflammation. The decoction prepared from the flowers of *A. arabica* Kotschy is externally dressed on the hemorrhoid nipples. Again, the decoction prepared from the aerial parts of the plant is mixed with real honey and the powder prepared by beating the plant, and it is used internally in diabetes. *A. arabica* Kotschy is bundled and hung in the house to make the environment smell nice. Drinking the infusion prepared from its flowers is used internally for the removal of kidney stones, urinary tract inflammation and prostate (20).

2.2. *Achillea millefolium L.*

Achillea millefolium L., known as "Civan perçemi" in Türkiye, is a plant that can grow between 10-100 cm. Stem shape of *A. millefolium* L. obtuse to angled. The petiole can be up to 5 cm and leaves are (2-) 3 pinnatisect. The inflorescence is 4-15 cm wide. Peduncle length is between 1-5 mm. The involucrum can be shaped from oblong to oval. The phyllaries may be oblong-lanceolate in shape and have brownish membranous margins. The petiole can be up to 5 cm, while the leaves are (2-) 3 pinnatisects. The inflorescence is 4-15 cm wide (Figure 1). Peduncle length is between 1-5 mm. The involucrum can be shaped from oblong to oval. The phyllaries may be oblong-lanceolate in shape and have brownish membranous margins. Ligulas are 4-6 and white. Disc flowers are 10-20 pieces. Flowering period is from June to September. Its habitat is mountain meadows. It can live in altitudes between 500-3450 m. The phytogeographic region which the plant belongs is the Irano-Turanian phytogeographic region (6,7).


Figure 4. *A. millefolium* L.

The distribution of *A. millefolium* L. in Türkiye is given in Figure 5, and its distribution in the world is given in Figure 6.
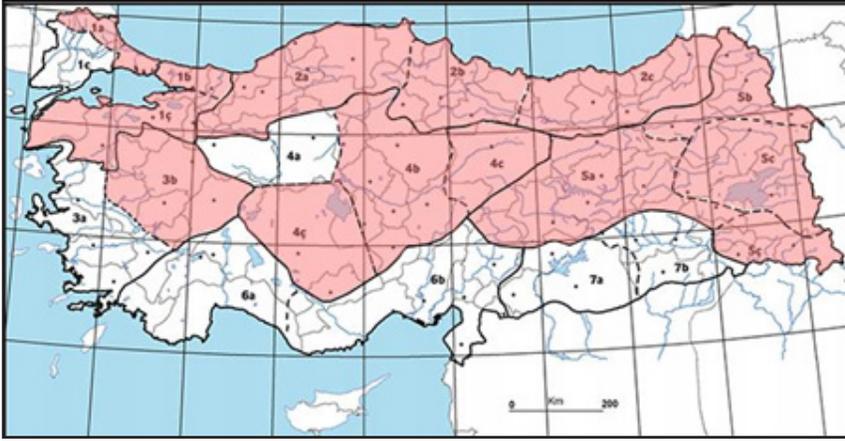
Figure 5. Turkey distribution map of *Achillea millefolium* L (7)
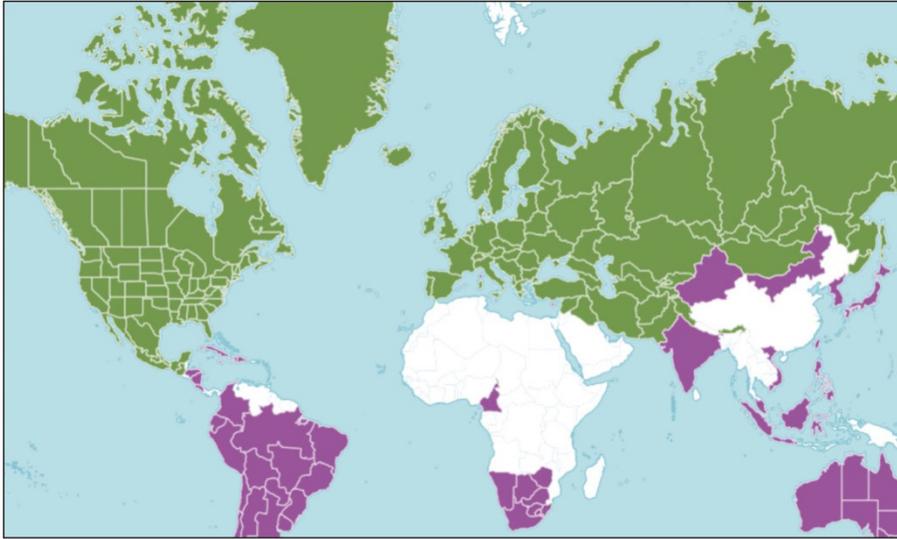
■ Native. ■ Introduced

Figure 6. World distribution map of *A. millefolium* L. (21)

In the studies, it observed that the *A. millefolium* L. has many local names as kurpotu (22), kılincok, kılıçotu (20), mor civanperçemi (23), akbaş otu, ayvadana, civanperçemi, göbek otu (24), anababa, ayvadenesi (19), civanperçemi (13), arı çiçeği (11), akbaşlı, binbiryaprakotu, barsamaotu, marsamaotu, kandilçiçeği (3) beyaz civanperçemi (10), hıpkesti (25).

All parts of the plant are prepared by decoction and used for toothaches (25). The tea, which is prepared by brewing the flowers, is drunk against urinary

tract disorders, menstrual pain and its regulation, abdominal pain and pain caused by gallbladder Stones (19). The flowers of the plant are dried and drunk as tea in case of sudden abdominal pain. Especially in external bleeding, the flowers are powdered on the bleeding area and moxibustion is applied (26). When the infusion prepared from the above-ground parts is consumed internally, it helps women in the menopausal period to pass the process easily and to relax the digestive system. It is used internally against hemorrhoids (23). After *A. millefolium* L. leaves are crushed by hand, they are made into pellets and applied to incisions or wounds. It is used externally for rapid healing of wounds without scarring, by stopping bleeding in cuts (20). Its flowers are steeped in hot water to lower sugar and cholesterol. If it is boiled and drunk, it dissolves kidney stones. Beekeepers put it near the bees to make honey. This plant, which is found fresh in the field, is fed to animals (22). When children are born, they are collected and crushed when they are still green so that they do not have an umbilical hernia. The plant is crushed while still green and used against umbilical hernia in newborn babies.It is placed on the navel of babies with the help of a cloth. Leaves and flowers are crushed and placed on a cloth and the person suffering from diarrhea is seated on it. It is brewed and drunk as tea to relieve gas pains (24). *A. millefolium* L. decoction is used in stomachaches (27). Decoction of *A. millefolium* L. used for earache respiratory diseases (28). Tea is made for respiratory diseases such as colds, flu, cough and bronchial asthma. (29).

It is used as a mouthwash in gingival diseases or as a compress in the residual swelling, wound, yeast after filtering. The boiled fresh plant is mixed with olive oil and used in the treatment of wormy wounds of animals. Widely used in the liquor and perfumery industries, the drug is also used to flavor milk. It is used against aphids in cultivated plants. It emits a sharp aromatic odor around it in sunny weather. In fact, it is necessary to collect flowers during the hours when the sun is most effective, because at that time its essential oils and healing power are at their peak (30). When wounds occur on the feet of animals, they boil and pour them on their feet. The paint is removed (31).

Artemisia absinthium L

Artemisia absinthium L. is an aromatic, perennial shrub-like herb with green-gray leaves and small yellow flowers and a bitter taste. The content of *A. absinthium* L. was first used as a medicine in 1792 by a French physician. Later, recreational alcoholic beverages were made from wormwood in some European countries in the 18th and early 19th centuries. These drinks became famous for

triggering psychotic events, and since intoxication and nervous disorders were observed in those who used these drinks, starting from 1908, it was forbidden to put this plant in drinks (3,32).

A. L., also called "acı peliotu", is a aromatic perennial shrub. Flowering stems are erect and up to 1 m high. Leaves 2-3 pinnatisect, lobes almost oblong, subacute, distinctly decurrent along segments, both faces of leaves grayish or whitish. Inflorescence is narrow or broad panicle (Figure 7). Bracts are similar to leaves but smaller. Capitula depressed-globose, many-flowered, 3-5 (-6) mm wide. The involucre is 1-3 mm long. Receptacle conspicuously pilose. Outer flowers are filiform, female. Inner flowers are hermaphrodite, fertile, corolla yellowish and glabrous. Flowering time is in June-September. It grows up to a height of 2600 m on stream banks, fields and steppe places (6).


Figure 7. *Artemisia absinthium* L.

The distribution of *A. absinthium* L. Türkiye is given in Figure 8, and its distribution in the world is given in Figure 9.

Figure 8. Türkiye distribution map of *A. absinthium* L. (33)
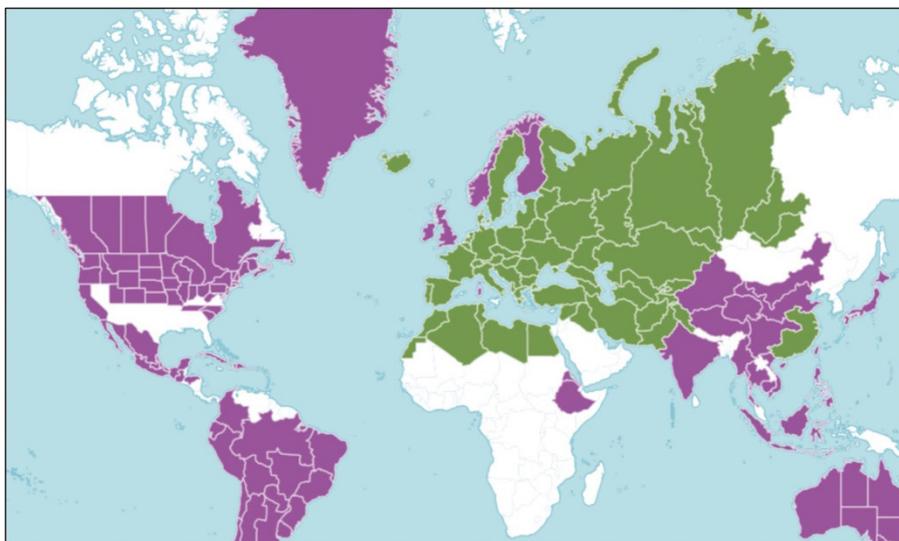
■ Native. ■ Introduced

Figure 9. World distribution map of *Artemisia absinthium* L. (34)

In the studies, it observed that the *A. absinthium* L. has many local names as; parlakot, yavşan, bitotu (26), mide otu (25), pelin (13,29), bire otu (16), acı pelin, büyük pelin, ak pelin, (3), mayasıl otu (9), bevüjana kuvi (35).

The decoction prepared from the leaf and stem of *Artemisia absinthium* L. is used for diabetes, abdominal pain and inflammation (25). It is used as an anthelmintic in humans and animals by preparing infusion tea from the aerial parts (13,36). The above-ground parts are applied to the cowhide as an insect

repellent (29). It is used as a repellent for insects such as lice and fleas, which are found in homes while the plant is in bloom and without drying (26). The decoction prepared from the plant is used as a sedative, antipyretic, diuretic, for headaches, asthma and diabetes. The aerial parts are burned and people who have a headache are kept in its smoke. The decoction prepared from the above-ground parts is used internally as a pain reliever and as a digester. The infusion prepared from the newly emerged leaves is used internally in asthma, as an antipyretic, in diabetes, as an appetite stimulant, as a potentiator. The decoction prepared from the aerial parts is used to prevent fleas from entering the barns. Insects are prevented from entering the house by placing the plant under the carpet, between the beds. Used as an igniter after the above-ground parts have dried. Used as an igniter after the aerial parts have dried (16). The decoction prepared from the whole plant is used for hemorrhoids (9). It is used as a stimulant, appetizer, antipyretic and diuretic. It also has worm-reducing and menstrual-inducing effects. However, these effects only occur at high doses. In these doses, it causes dangerous poisoning due to the essential oils it carries (3). With the distillation process, it yields a dark green oil with a strong odor of the plant and a bitter taste. The essential oil of the plant is used traditionally, as well as for Anorexia nervosa, backache, cold sores, improvement of mental state, insect and spider bites, jaundice, labor pains, neonatal jaundice, skin wounds, stomach ailments (32). The above-ground part is boiled and drunk for the treatment of shortness of breath and diabetes. The aerial parts are boiled and drunk for the treatment of shortness of breath and diabetes (35).

2.4. Tanacetum balsamita L.

Tanacetum balsamita L. whose Turkish name is "gümüşdüğme" is a Rhizomatous perennial herb. The stem is 35-80 cm long and s sparsely pubescent. Stem is branched above. Basal leaves are ovate-elliptic and 12-20 cm x 1.5-5 cm in size. The apex of leaves is acute or subobtuse; base of leaves cordate, turncate or cuneate. Basal leaf margins crenate-serrate and adpressed-pubescent on both surfaces. Stem leaves are similar to basal leaves but smaller in size. Lower stem leaves shortly petiolate, becoming sessile above. Capitula usually numerous, (3-)30-100 in loose terminal corymbs. The involucrum is 5-8 mm wide. The phyllaries are 3-series, lanceolate or oblong, with light or dark margins. Ray flowers 12-15 or absent, ligules white, disc flowers yellow. Flowering period is between July and September. It lives in moist areas. Sometimes it is cultured. It grows at altitudes between 1100-3000 m (6).

Figure 10. *T. balsamita* L.

The distribution of *T. balsamita* L. Türkiye is given in Figure 11, and its distribution in the world is given in Figure 12.
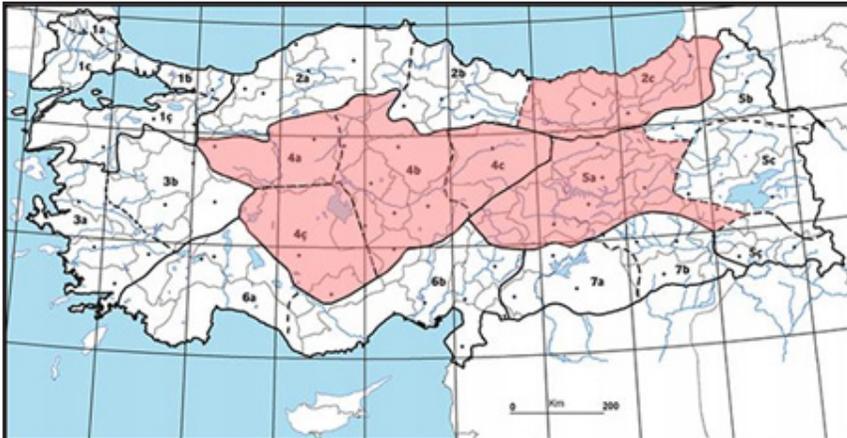
Figure 11. Türkiye distribution map of *Tanacetum balsamita* L. (37)
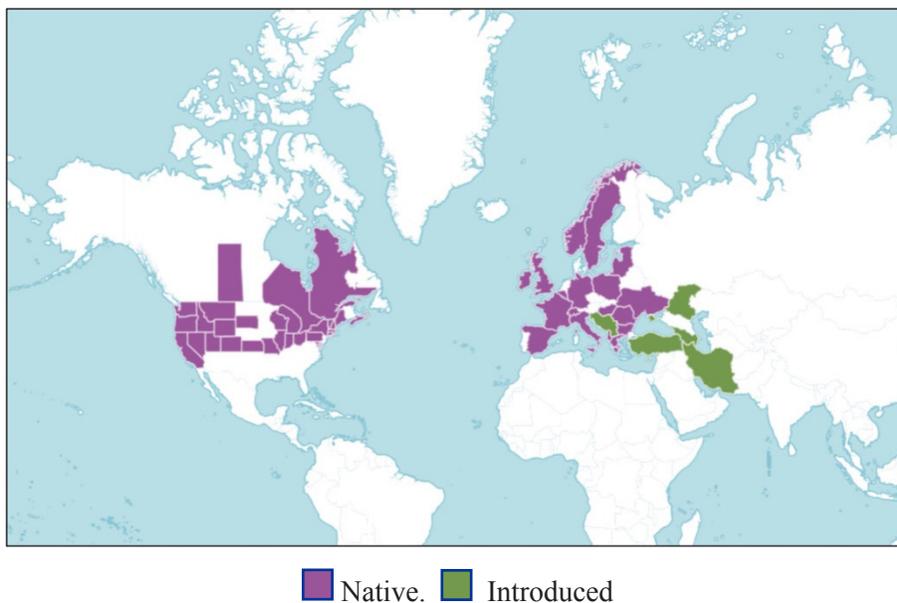
■ Native. ■ Introduced
Figure 12. World distribution map of *T. balsamita* **L (38)**

In the studies, it observed that the *T. balsamita* L. has many local names as; marsuvanotu (3,13), boz boz, kılıç otu (25), gıyakeçk (35).

Its flowering branches are used as an infusion, as a diuretic, gas-reducing agent, stomach and gall bladder stones (3). Decoction from the leaves and stem of the plant is used in the treatment of deep lesions, in the preparation of creams for rheumatism and lesions, as a menstruation regülatör (25). Infusion prepared from the plant as a stimulant, antipyretic and diuretic; Used for stomach, abscess and headache (27). The porridge of the leaf part of the plant is placed on the wounds and inflamed places on the body (35).

2.5. *Cichorium intybus L.*

C. intybus L. is called as "Hindiba" in Turkish is a roughly hairy or glabrous perennial. It is stout tap rooted. The stem is stiff, grooved and 20-100 cm. Basal leaves are shortly petiolate. Cauline leaves similar basal leaves but they are sessile. The capitula is 2.5-3.5 cm broad. The outer phyllaries are ovate, while the inner ones are lanceolate and hairless. Flowering period is between (April) June to September. They live in fields, meadows and wet places. Its altitudes are from sea level to 3050 m (6).

Figure 13. *C. intybus* L. (39)

The distribution of *C. intybus* L. Türkiye is given in Figure 14, and its distribution in the world is given in Figure 15.
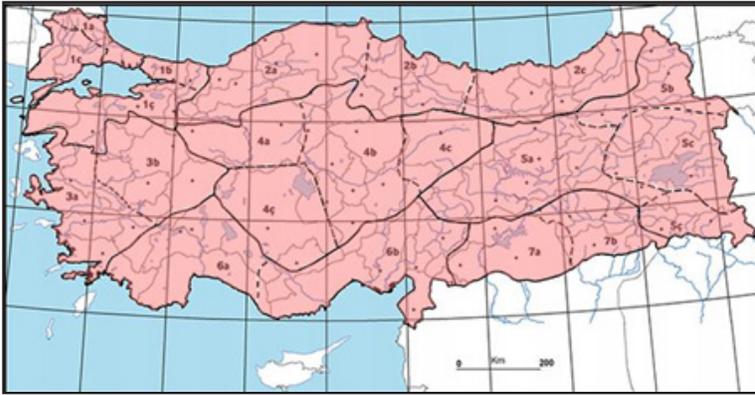
Figure 14. Türkiye distribution map of *C. intybus* L. (40)
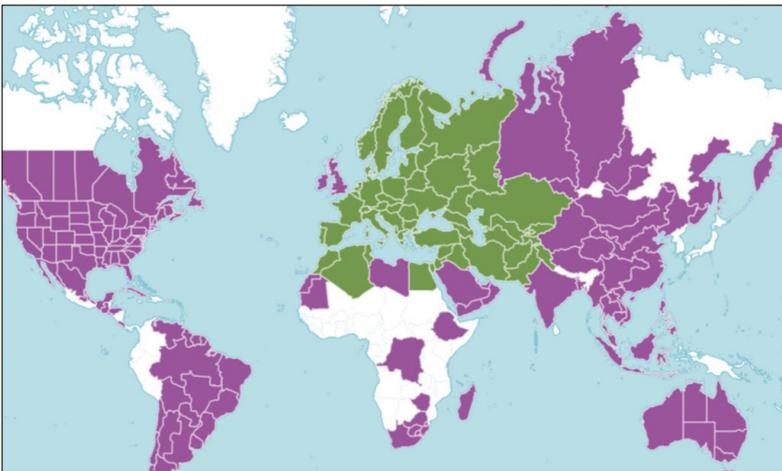
Native. Introduced

Figure 15. World distribution map of *Cichorium İnthybus* L. (41)

In the studies, it observed that the *C. İnthybus* L. has many local names as; kanej, şembelk, (10), kaniş (35), acı hindiba (24), hindibağ (27), çiftlik otu, güneyik, acı güneyik (19), tehli, taliye, tali (1), ham sütlüvan, gürlük otu, karakovuk, kara hindiba, mayasıl otu, mavi hindiba, radika, radik, sakız otu, sakız çiçeği, sakızlık otu, sütlü ot, yabani hindiba, talışk (5), cırtlankuş, cızdankuş, çırtlangu (16), gıcıbıcı, çekçekon, çini çiçeği, talişk, badik otu, karakavuk, acıgıcı, acı marul, çatlangaç süpürgesi, çitlek otu, çatlak otu, çıtlık, ayakçak otu, yer sakızı, eşek sakızı, kaniş, acı hindibağ, ham sütlüvan, eşek karakavuğu, çukur otu (15).

The sap of the plant is used in skin disorders such as fungus, eczema, warts and psoriasis. In addition, the dried plant is boiled in water for 5 minutes and drunk like tea; It is used against abdominal pain, constipation, prostate cancer and diarrhea (10). The milk flowing from the branch of the plant is dripped into the water and consumed to relieve the pain in the abdomen. After the aerial parts are dried and boiled in water, the juice is drunk for prostate disease. The aerial part of the plant is boiled in water and the water is drunk to lower blood pressure (35). Food is made using its leaves (24). It is used in the treatment of liver disorders, cleaning the biliary tract (42). Fresh branches of *Cichorium intybus* L. are used to treat dermatitis. (27). If the aerial parts of the plant are boiled and drunk, it will be beneficial in gallbladder and liver diseases, and it has diuretic properties. It is appetizing and cleans the blood. (22). It is used internally for diarrhea, blood sugar, liver diseases and diuretic (29). Gum is obtained from the root of *C. intybus* (20). Its leaves are consumed raw to reduce kidney stones and relieve pain in the kidneys. Its roots are collected before blooming and roasted and consumed for nutritional purposes and against stomachache. Its roots are stringed and dried and consumed during the winter. It is consumed against diabetes. (19). In the boil, the seed is crushed by pounding and wrapped externally (43). Tea in the form of infusion prepared from the dried flower is used as liver cleanser, appetizer, sugar reducer and headache reliever (12). The ash, which is completely burned, is stored and applied externally on the herpes. It is said that the ash obtained by burning the whole plant together with the sandal was used externally in the treatment of baldness by soaking it in the past. (16). The milk in the root and stem is rubbed on the wounds (15). The aerial parts are used for prostate cancer (10).

Table 1. Overview of studies about antibacterial properties of plants

Essential oil/extract from	Microorganisms	Method	MIC/Inhibition zone	Reference
Achillea arabica (essential oil)	*Staphylococcus aureus, Listeria monocytogens, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis*	Broth microdilution	60.0-120.0 µl/ml	(44)
Achillea arabica (Ethanol extract)	*Klesiella pneumoniae, Enterobacter cloacae, Salmonella typhimurium, Staphylococcus epidermis, Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Klebsiella oxytoca, Streptococcus pyogenes, Pseudomonas aeruginosa, Candida albicans*	Agar well diffusion	8-24 mm	(45)
Achillea arabica (Ultrasound extract)	*Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Candida albicans*	Broth microdilution	0.6-20 mg/mL	(46)
Achillea arabica (Maceration)	*Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Candida albicans*	Broth microdilution	1.2-40 mg/mL	(46)
Achillea arabica (Aqueous extract)	*Staphylococcus aureus, Clinical Strain MRSA, Klesiella pneumoniae*	Broth microdilution	25-50 mg/mL	(47)
Achillea arabica (n-hexane extract)	*Enterococcus faecalis*	Broth microdilution	125 µl/ml	(48)
Achillea arabica (methanolic extract)	*Staphylococcus aureus*	Broth microdilution	162.5-225 µl/ml	(48)
Achillea arabica (essential oil)	*Achromobacter piechaudii, Bacillus pumilus, Euoniticellus intermedius, Erwinia caratovora, Erwinia chrysanthemi, Erwinia rhapontici, Flavobacter sp., Pantoea agglomerans, Pseudomonas aeruginosa, Pseudomonas cichorii, Pseudomonas syringae, Xanthomonas axonopodis, Xanthomonas campestris*	Agar well diffusion	125-500 µl/ml	(49)
Achillea arabica (essential oil)	*Salmonella enteritidis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klesiella pneumoniae, Enterobacter aerogenes, Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis*	Broth microdilution	2629.2-42000 µg/ml	(50)
Achillea arabica (maceration)	*Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis*	Broth microdilution	0.6-10 mg/mL	(51)
Achillea arabica (essential oil)	*Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Clostridium perfringens,*	Broth microdilution	0.15-72.0 mg/mL	(52)

	Mycobacterium smegmatis *Candida albicans*			
Achillea arabica **(essential oil)**	*Staphylococcus aureus,* *Bacillus cereus,* *Escherichia coli,* *Salmonella enteritidis,* *Pseudomonas aeruginosa,* *Candida albicans*	Broth microdilution	0.62-20 mg/mL	(53)
Achillea arabica **(methanolic extract)**	*Staphylococcus aureus,* *Streptococcus pyogenes,* *Enterococcus faecalis,* *Escherichia coli,* *Pseudomonas aeruginosa,* *Acinetobacter sp.,* *Proteus mirabilis,* *Klebsiella sp.,* *Streptococcus pneumoniae*	Micro-well dilution	12.5-50 mg/mL	(54)
Achillea millefolium **(essential oil)**	*Staphylococcus aureus,* *Bacillus cereus,* *Escherichia coli,* *Salmonella enteritidis,* *Pseudomonas aeruginosa,* *Candida albicans*	Broth microdilution	0.62-20 mg/mL	(53)
Achillea millefolium **(essential oil)**	*Bacillus cereus,* *Enterococcus faecalis,* *Staphylococcus aureus,* *Escherichia coli,* *Pseudomonas aeruginosa,* *Proteus mirabilis,* *Salmonella typhimurium,* *Citrobacter freundii,* *Candida albicans,* *Aspergillus fumigatus*	Broth microdilution	4.5-20 µg/mL	(55)
Achillea millefolium **(methanolic extract)**	*Staphylococcus aureus,* *Bacillus cereus,* *Escherichia coli,* *Staphylococcus epidermidis,* *Salmonella typhimurium.*	Agar dilution	50-100 µg/mL	(56)
Achillea millefolium **(essential oil)**	*Staphylococcus aureus,* *Streptococcus pneumoniae,* *Bacillus cereus,* *Acinetobacter lwoffii,* *Enterobacter aerogenes,* *Escherichia coli,* *Klebsiella pneumoniae,* *Clostridium perfringens,* *Mycobacterium smegmatis,* *Candida albicans,* *Candida krusei*	Broth microdilution	4.5-72.0 µg/mL	(57)
Achillea millefolium **(essential oil)**	*Bacillus cereus,* *Enterococcus faecalis,* *Staphlococcus aureus,* *Escherichia coli,* *Pseudomonas aeruginosa,* *Proteus mirabilis,* *Salmonella typhimurium,* *Citrobacter freundii*	Broth microdilution	2.5-5.0 µg/mL	(58)
Achillea millefolium **(essential oil)**	*Staphylococcus aureus,* *Staphylococcus epidermidis,* *Bacillus cereus,* *Entrococcus faecalis,* *Escherichia coli,* *Pseudomonas aeruginosa,* *Klebsiella pneumonia,* *Salmonella typhymorium,* *Shigella dysentria*	Agar dilution	12.6-112 µg/mL	(59)
Achillea millefolium **(essential oil)**	MRSA, *Staphylococcus aureus,* *Pseudomonas aeruginosa,* *Escherichia coli,* *Bacillus cereus*	Agar well diffusion	10-21 mm	(60)

Achillea millefolium (maceration)	*Escherichia coli,* *Pseudomonas aeruginosa,* *Staphylococcus aureus,* *Salmonella enteritidis,* *Aspergillus niger,* *Candida albicans*	Disc diffusion	15-17 mm	(61)
Achillea millefolium (methanolic extract)	*Escherichia coli,* *Staphylococcus aureus,* *Pseudomonas aeruginosa,* *Salmonella typhymurium,* *Serratia marcescens,* *Klebsiella pneumonia*	Broth microdilution	0.25-5 µg/mL	(62)
Artemisia absinthium (essential oil)	*Staphylococcus aureus,* *Bacillus licheniformis,* *Micrococcus luteus,* *Enterobacter xiangfangensis,* *Escherichia fergusonii,* *Pseudomonas aeruginosa,* *Candida albicans,* *Candida parapsilosis,* *Aaspergillus parastiticus*	Broth microdilution	4-25 µg/mL	(63)
Artemisia absinthium (ethanolic extract)	*Escherichia coli,* *Bacillus subtilis,* *Pseudomonas syringola*	Disc diffusion	0.4-1.7 mm	(64)
Artemisia absinthium (methanolic extract)	*Staphylococcus aureus,* *Salmonella typhymurium,* *Shigella flexneri,* *Pseudomonas aeruginosa,* *Escherichia coli*	Broth dilution	1.3-6.5 mg/mL	(65)
Artemisia absinthium (ethanolic extract)	*Trypanosoma b. brucei,* *Trypanosoma cruzi,* *Plasmodium falciparum,* *Microsporum cani,* *Candida albicans,* *Escherichia coli,* *Staphylococcus aureus*	Broth microdilution	1-64 µg/mL	(66)
Artemisia absinthium (essential oil)	*Escherichia coli,* *Staphylococcus aureus,* *Staphylococcus epidermidis,* *Candida albicans,* *Cryptococcus neoformans,* *Trichophyton rubrum,* *Microsporum canis,* *Microsporum gypseum*	Agar diffusion	5-28 mm	(67)
Artemisia absinthium (essential oil)	*Staphylococcus epidermidis,* *Micrococcus flavus,* *Micrococcus luteus,* *Bacillus subtilis,* *Aspergillus niger,* *Aspergillus fumigatus,* *Penicillium chrysogenum*	Tube dilution	25-238 µg/mL	(68)
Artemisia absinthium (essential oil)	*Salmonella enteritidis,* *Escherichia coli,* *Pseudomonas aeruginosa,* *Proteus mirabilis,* *Klebsiella pneumonia,* *Enterobacter aerogenes,* *Bacillus cereus,* *Staphylococcus aureus,* *Enterococcus faecalis*	Micro-well dilution	647.4-5187.8 µg/mL	(50)
Artemisia absinthium (hydroalcoholic extract)	*Staphylococcus aureus*	Broth macrodilution	3×10^2 (experiment), 7×10^6 (control) CFU/wound	(69)
Artemisia absinthium (essential oil)	*Staphylococcus aureus,* MRSA *Listeria monocytogenes,* *Fusarium graminearum,* *Fusarium culmorum,* *Fusarium oxysporum,* *Sclerotinia,* *Rhizoctonia solani*	Disc diffusion	11.11-46.82 mm	(70)

Artemisia absinthium (essential oil)	*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa, Aeromonas hydrophila, Listeria monocytogenes, Bacillus cereus, Aspergillus flavus, Aspergillus niger, Candida albicans*	Disc diffusion	10-20 mm	(71)
Artemisia absinthium (methanolic extract)	*Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhimurium, Saccharomyces cereviciae, Bacillus cereus, Candida albicans, Streptococcus thermophilus, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus hominis, Enterobacter cloaceae, Escherichia coli, Proteus mirabilis, Providencia alcaliaciens, Acinetobacter lwoffi, Pseudomonas pseudoalcaligenes, Pseudomonas fluorescens, Pseudomonas putida, Flavobacterium indologenes, Yersinia enterocolitica, Penicillium brevicompactum, Trichothecium roseum*	Disc diffusion	6-19 mm	(72)
Artemisia absinthium (essential oil)	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Acinetobacter baumannii*	Broth dilution	0.5-5 mg/mL	(73)
Artemisia absinthium (essential oil)	*Streptococcus salivarius, Streptococcus sanguinis, Streptococcus mitis, Streptococcus mutans, Streptococcus sobrinus, Enterococcus faecalis, and Lactobacillus casei*	Broth microdilution	62.5-1000 µg/mL	(74)
Artemisia absinthium (essential oil)	*Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis, Staphylococcus epidermidis, Enterobacter cloaceae, Escherichia coli, Klebsiella pneumonia*	Agar well diffusion	7-25 mm	(75)
Cichorium intybus (ethanolic extract)	*Bacillus subtilis, Styphyloccus aureus, Bacillus atrophoeus, Salmonella typhi, Klebsella pneumoniae, Escherichia coli, Candida albicans, Rhizopus sp., Aspergillus sp.*	Disc diffusion	10.5-22.5 mm	(76)
Cichorium intybus (methanolic extract)	*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Listeria monocytogenes, Escherichia coli O157: H7, Citrobacter freundii, Salmonella enteritidis, Enterococcus aerogenes, Klebsiella pneumoniae*	Disc diffusion	6.81-14.84 mm	(77)
Cichorium intybus (Aqueous extract)	*Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa,*	Agar well diffusion	70-100 mL	(78)

	Escherichia coli			
Tanacetum balsamita (essential oil)	Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enteritidis, Salmonella typhimurium, Yersinia enterocolitica, Shigella sonnei, Pseudomonas aeruginosa	Broth microdilution	1-32 μL/mL	(79)
Tanacetum balsamita (essential oil)	Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Candida globrata, Candida tropicalis	Agar well diffusion	13-17 mm	(80)
Tanacetum balsamita (essential oil)	Bacillus subtilis, Bacillus pumilus, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Saccharomyces cerevisiae	Broth microdilution	0.93-15 mg/mL	(81)

3. Conclusion

It is known that plants are used for medicinal purposes as well as being consumed as food by the local people for many years. The experiences of many years, supported by science, have been revealed in many studies. There are many studies on *Achillea arabica*, *Achillea millefolium*, *Artemisia absinthium*, *Tanacetum balsamita* and *Cichorium intybus*, which have been used by the local people for many years in Kars. We have compiled studies in which these plants, which have been examined in many medical terms, are only considered from an antimicrobial point of view. These plants appear to have antimicrobial properties. Although many positive medicinal effects of plants have been shown, their potential negative effects should also be evaluated. More comprehensive studies on these plants may pave the way for the pharmaceutical use of plants for therapeutic purposes.

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ACTIVE COMPOUND OF PISTACHIO

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1.   Introduction

The genus Pistacia belongs to the cosmopolitan family Anacardiaceae (Table 1), comprising about 70 genera and more than 600 species. Species of the genus Pistacia are resinous trees that are characterized as xerophytic trees and can reach 8-10 m in height. *Pistacia lentiscus* L., *P. atlantica* Desf., *Pistacia terebinthus* L., *Pistacia vera* L., and *Pistacia khinjuk* Stocks. distributed from the Mediterranean basin to Central Asia (1,2). *Pistacia vera*, known commonly as "pistachio" is cultivated in the Middle East, United States, and Mediterranean countries (3).

Table 1. Scientific classification of *P. vera*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Anacardiaceae
Genus	Pistacia
Species	*Pistacia vera* L.

Pistacia species are used in food industry, for example, the seed obtained from the pistachio fruit (Fig 1) is used as a snack and traded around the world (4).

Records of the consumption of pistachios as food date back to 7000 BC (4). In traditional Iranian medicine, different parts of *P. vera* have long been used as beneficial medicines for different diseases (5,6).

Fig 1. *P. vera* fruit

Pistachio is considered a very important nutritional product and a good source of unsaturated fatty acids. Pistachios are very valuable for their nutritional, sensory and health properties. In addition to containing high levels of unsaturated fatty acids, it is a good source of protein, vitamins, minerals, fiber and antioxidants (7-9).

Bioactive compounds, which are mostly intermediates consisting of the products of primary metabolism, are phytochemicals that develop as a result of secondary metabolic activities of plants, cannot be consumed as food, but have beneficial effects for human health (10).

These bio-active compounds act as anti-degenerative, anti-allergenic, anti-inflammatory, antimicrobial, antithrombotic (preventing blood clotting), anticarcinogen, antiatherogen (preventing atherosclerosis), antiulcer and vasodilator (blood vessel dilator) agents used against many diseases (11-13).

There are various bioactive components that show phytochemical activities in different parts of pistachios as given in Table 2. These are summarized below.

2. Bioactive Compounds

2.1. Terpenes

Terpenes and terpenoids (modified terpenes) are the main components of essential oils in many plant species. Their classification is usually based on isoprene units. Essential oils contain mostly mono- and triterpenoids (14).

Monoterpenoids. It is one of the main components found in different parts of Pistacia species, including leaves, resin, mature and immature fruits, galls, leaf buds, twigs and flowers. (15,16). The major chemical components in the essential oil are hydrocarbon and oxygenated monoterpenes, and among the hydrocarbon monoterpenes, α-pinene has been reported to be one of the main compounds of *P. vera* (15,17,18). In addition to α-pinene, limonene, α-terpinolene and okimen from the fruit and leaves of *P. vera* (19); Some of the other monoterpenes identified as effective antibacterial components of these essential oils include camphene, limonene and carvacrol from *P. vera* resin (15).

Diterpenoids. Diterpenoids are found in trace amount in Pistacia species. Abietadiene and abietatrien were detected in the essential oil of *P. vera* resin (15).

Triterpenoids. The resin of Pistacia species is characterized by penta and tetracyclic triterpenes. Especially in the acidic fractions of *P. vera* resin, there are triterpenes such as masticadienonic acid, masticadienolic acid, morolic acid, oleanolic acid, ursonic acid and their derivatives (20-22).

2.2. Phenolic compounds

Phenolic compounds are secondary metabolites synthesized during aromatic amino acid metabolism in plants. Capability to condense up to eighty monomer compounds, forming a complex with proteins, and soluble in water have been among the physicochemical properties of these compounds (23). They are present in all parts of plants at different levels and contribute to the taste, odor and color properties of plants and also they have antimicrobial and antioxidant effects and may cause inhibition of different enzymes. Phenolic compounds are characterized as natural players of antioxidant metabolism and bind free radicals or form chelates with metals (23,24). This effect increases as the number of OH groups in the phenol ring increases (23) and has the ability to delay, slow down or prevent oxidation at low concentrations, and to remain in a stable form when converted to free radicals (25,26). In addition to its antioxidant

properties, it also has antiallergen, antimutagen, anticarcinogen, antiglycemic, anticholesterol, antimicrobial, anti-inflammatory, antithrombotic, vasodilator and calming properties. So they are used in the cosmetics, pharmaceutical and food industries (27-29). Studies have reported that individuals who include foods with high phenolic content in their diets have a reduced risk of coronary artery disease (30).

Phenolic compounds increase the activity of enzymes responsible for the inhibition of cancerous cells and prevent the development of nitrosamine, which has an important role in tumor formation. It also regulates the ion balance in the intestinal flora and the pH in the environment. By assuming the protection of the integrity of the intracellular matrices, they enable the cell to resist environmental effects (31). Phenolic compounds are classified according to the number of contained phenol rings and connected structures with these rings, and in general; flavonoids (anthocyanidins, flavones and flavonols, flavanones, flavanols (catechins) and isoflavones) are grouped as phenolic acids, stilbenes and lignans (32).

Gallic acid, catechin, epicatechin, and gallic acid methyl esters have been identified in the seed and hull of *P. vera* (33-35). Monounsaturated and saturated cardanols were detected in *P. vera* seeds (36).

There are flavonoid compounds in different parts of the pistachio. Aringenin, eriodyctyol, daizein, genistein, quercetin, kaempferol, apigenin, luteolin and quercetin-3-O-rutinoside are the main bioactive components of the fruit (33). Also, Cyanidin-3-O-glucoside, cyanidin-3-galactoside, and quercetin-3-O-rutinoside are the main anthocyanins of *P. vera* fruit (33,37,38). It has been determined to be rich in especially phenolic active metabolites such as eriodicthiol, quercetin, naringenin and apigenin in methanol extract of *P. vera* hull (39). On the other hand, its dichloromethane and methanol extracts were found to contain myristic acid, palmitic acid, margaric acid, stearic acid, elaidic acid, oleic acid, linoleic acid, as well as anacardic acid derivatives and tocopherols (40). The presence of anacardic acid derivatives and shikimic acid was given in the hull ethanolic extract (41).

2.3. Fatty Acids and Sterols

Fatty acids are straight chain carboxylic acids even number of carbon atoms and chain lenght varying between 4 and 28 carbons. The oil content in *P. vera* seed is approximately 50-60%, although the main fatty acid component is oleic acid (42-44).

It has been revealed that the essential oil of *P. vera* hull and resin is rich in α-pinene (45). In addition, in another study with *P. vera* hull, the main bioactive components were determined as 4-karen, α-pinene, limonene and 3-karene (46).

Table 2. Bioactive Compounds in Different Parts of Pistachios

Part	Metabolite	Ref
Nut	Cyanidin-3-O-gal	(61)
	Procyanidin B1	
	oleic acid	(42-44)
	luteolin	
	apigenin	
	kaempferol	(33)
	genistein	
	daizein	
	eriodyctyol	
	naringenin	
	cardanol	(20)
	Gallic acid methyl ester	
	Epicatechin	(33-35)
	Anacardic acid	(62)
	Gallic acid	
	Catechin	(33)
	Quercetin	
	α-terpinolen	(17)
	α-Pinene	
	Limonen	(63)
	Myrcene	

Hull	Gallic acid	(33)
	Catechin	
	Gallic acid methyl ester	(64)
	Epicatechin	
	Palmitic acid	(65)
	Oleic acid	
	Linoleic acid	
	Beta-Sitosterol	
	Quercetin	
	Myrcene	(66)
	3-karen	
	Pinokarvon	
	α-terpinolen	
	Kamfen	
	α-Pinene	(67)
	D-limonen	
	Shicimic acid	(41)
	Anacardic acid	
Shell	Ursolic acid	(69)
Stem	L-bornyl acetate	(68)
	3-carene	
	Sabinene	
	α-Pinene	(69)
	Kamfen	
	β-Pinene	
	Limonen	
	Camphene	
	Kamfor	

Leaf	α-Pinene	(45)
	Myrcene	
	α-terpinolen	(17)
	bornil asetat	
	ocimene	(19)
	limonene	
resin	camphene	(70)
	limonene	(15)
	carvacrol	
	abietadine	
	abietatrine	
	masticadienolic acid	(20-22)
	masticadienonic acid	
	morolic acid	
	oleanolic acid	
	ursonic acid	

3.   Pharmacological Aspects

3.1. Antioxidant Activity

P. vera fruit has a strong antioxidant activity (47). The lipophilic extract from the *P. vera* fruit showed lower antioxidant potential than the hydrophilic extract (7). One study showed that *P. vera* hull had a better antioxidant activity compared to seed due to the higher content of antioxidant phenolic compounds in the hull (33). Antioxidant activity has also been reported from other parts of *P. vera* (48). It has been determined that 4-karen, α-pinene, limonene and 3-karene metabolites, which are the main bioactive components of *P. vera* hull which have antioxidant activity (46,7).

3.2. Antimutagenic Activity

Gallic acid found in the fruit and hull parts of *P. vera* has been found to induce inhibitory activity against mutagenicity and have genotoxic activity (49,50).

3.3. Antimicrobial and Antiviral Activities

P. vera have demonstrated significant antibacterial activity against various Gram positive (*Staphylococus aures*, *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) (51). Antimicrobial activity of *P. vera* major constituent, α-pinene, against *Helicobacter pylori* were recorded (18,52) and rest of bioactive components of *P. vera* hull, 4-karen, α-pinene, limonene and 3-karene metabolites have antifungal activity against to three Candida species (*C. albicans*, *C. parapsilosis* ve *C. alabrata*) (46). Extract from *P. vera* branch had significant inhibitory activity against *Leishmania donovani* and leaf extract inhibited *Plasmodium falciparum* without cytotoxicity on mammalian cells (53). In addition, nut and seed extracts showed significant antiviral activity (54).

3.4. Anti-Inflammatory and Antinociceptive Activity

Masticadienonic acid and masticadienolic acid isolated from *P. vera* resin have been found to have anti-inflammatory activity (55). In addition, Oleoresin and leaf extract from *P. vera* have also been reported to exhibit significant anti-inflammatory and antinociceptive activity (48).

3.5. Cytotoxic and Antitumor Activity

Oleoresin obtained from *P. vera* showed moderate cytotoxic activity against breast, hepatocellular, cervical cancer cells and normal melanocytes (56), as well as α-pinene found in *P. vera* leaf, resin and fruit. It has been reported that limonene, one of the substances found in leaves, stems, hulls and fruits, did not show cytotoxic effects against J774 mouse BALB/C monocyte macrophage cells (57).

3.6. Effects on Liver and Serum Biochemical Parameters

The United States Food and Drug Administration (FDA) stated that the use of pistachios in diets can reduce the risk of heart disease due to the fact that the saturated fat content is very low (58).

It was determined that methanolic and cyclohexane extracts from *P. vera* fruits showed beneficial effects on High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL) and aortic intimal thickness in the rabbit atherosclerosis model, while methanolic extract showed a significant reduction in aortic surface lesions (59).

Positive changes in lipid profile were recorded after three-weeks use of *P. vera* nuts in patients with moderate hypercholesterolemia. The decrease in triglyceride and LDL levels was not significant (60) of *P. vera* nuts in patients with moderate hypercholesterolemia**.**

In addition, it was determined that 4-karen, α-pinene, limonene and 3-karen metabolites, which are the main bioactive components of *P. vera* hull, decrease the amount of lactate dehydrogenase (46).

4.   Conclusion

Plants have the ability to synthesize bioactive substances that they use as defense molecules, and many studies carried out today aim to isolate and identify these compounds with important pharmacological activities for the treatment of human diseases. Determining the pharmacological activities of many identified active metabolites such as antitumor, antioxidant and antibacterial encourages researchers to conduct more comprehensive studies on the potential applications of these components in human health. Among these researches carried out today to find new molecules from natural sources, there is *P. vera*, which contains quite a lot of bioactive components. This review of existing research on Pistachio, emphasizing pharmacological studies on active metabolites, presents scientific evidence of the biological activity of this plant, making it a valuable medical resource.

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AN OVERVIEW OF SILYMARIN EFFECTS ON HEALTH

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1.   Introduction

*S*ilybum marianum is the scientific name for silymarin, a polyphenolic flavonoid (1). Milk thistle, a member of the Compositae / Asteraceae family, is the source of *Cardus marianus* (2). Seeds of *Silybum marianum* are used to treat liver and gall bladder problems, as well as toxin poisoning (3). It's also been used for ages to treat bug stings, mushroom poisoning, and snake bites (4). *Silybum marianum* is grown in the eastern United States and Southern America, North America, and southern and western Europe (5). Its use as a medicinal plant, It was first reported by Theophrastus at the beginning of BC 4. the century (6). Silymarin is prevalent in extracts made from *S. marianum* seeds. Silymarin extract is made from the plant's seeds, which account for roughly 4-6 percent of the total. About 70-80% of the active extract of the plant contains silymarin, while 20-30% of the polymeric and oxidized polyphenolic and chemically unidentified fraction consists of flavonolignans. Silybin or silibinin constitutes 90% of the main components showing biological activity. Isosilybin, dehydrosilybin, silychristin, silydianin, and taxifolin are some of the other silymarin components discovered in *Silybum marianum* seeds. Silybonol, myristic, oleic, palmitic, and stearic acids, as well as betaine hydrochloride, are all potentially active components of the plant (7,8).
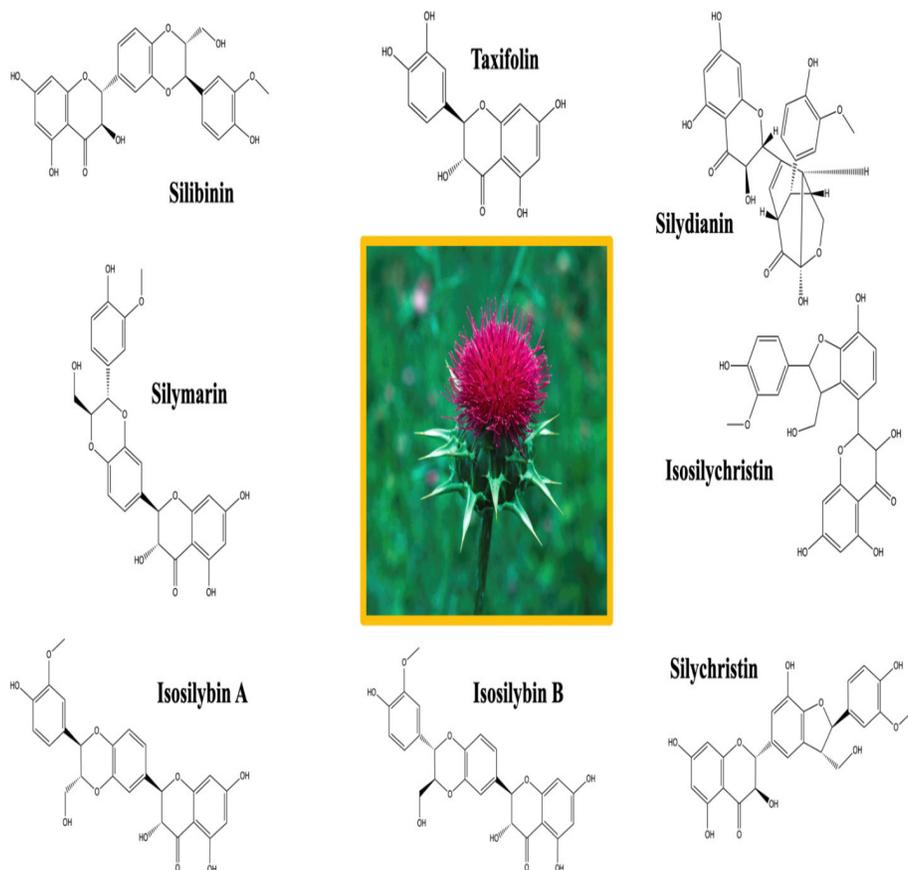
Figure 1. Chemical structures and active compounds in S. marianum (9).

Silymarin shows its antioxidative effect by removing free oxygen radicals and inhibiting lipid peroxidation (10). In addition, silymarin has biological functions such as antifibrotic, antilipid-peroxidative, anti-inflammatory, regulation of cell membrane permeability and regeneration of liver cells(11). It's commonly used in the treatment of liver problems, and it's also been shown to protect other organs and tissues (12). In this section, the miraculous effects of silymarin on other diseases, organs and tissues will be discussed. that is, in this section, we tried to focus on the importance and protective functions of silymarin in some health effects.

2.   Health effects of silymarin

Silymarin has been used for years in the treatment and prevention of various diseases. Silymarin has various protective effects such as reducing insulin

resistance, regulating blood pressure and lipid profile, as well as antioxidant and cytoprotective effects. It is known to have positive effects on the heart, kidneys and testis, especially on the liver. It also plays an important role in neurodegenerative diseases and cancer.

2.1. Protective Effect of Silymarin on the Liver

Its capacity to maintain the membrane by limiting lipid peroxidation and raising intracelar glutathione content explains its antioxidant effectiveness against free radicals. Silymarin has also been demonstrated to prevent the formation of free radicals such superoxide anion and nitric oxide. It is reported that silymarin reduces pro-apoptotic bax protein levels thanks to its liver protective function (13). It is also said to boost ribosomal protein synthesis by activating RNA polymerase I, making it useful in hepatocyte regeneration (14). Ferro et al.,(2020) discovered that silymarin lowers transaminase levels in people with nonalcoholic fatty liver disease and that long-term treatment reduces fibrosis in nonalcoholic steatohepatitis and may even stop the progression of liver disease (15). The glycogen phosphorylase enzyme activity in the ischemia group was substantially lower than in the control group, according to a study looking into the effects of silymarin on liver cell damage caused by ischemia (16). In a mouse study of acetaminophen-induced hepatotoxicity, researchers discovered that mice administered acetaminophen had a larger exposure to reactive oxygen species in the liver, but those given silymarin had a considerably lower impact. As a result, it is said to significantly reduce liver damage (17). In another study conducted in mice, it was reported that in the study induced with D-galactose/lipopolysaccharide, by showing positive results in antioxidative and anti-inflammatory parameters in the group treated with silymarin, preventing liver damage(18). Ferro et al.,(2020) discovered that silymarin lowers transaminase levels in people with nonalcoholic fatty liver disease in a study. In recent years, El Rabey et al.,(2021) reported that their research on CCl4-induced liver damage in rats demonstrated that silymarin is useful in protecting rats from CCl4 hepatotoxicity due to its antioxidant activities (19).

2.2. Protective Effect of Silymarin on Kidney

Ferro et al.,(2020) discovered that silymarin lowers transaminase levels in people with nonalcoholic fatty liver disease in a study. Silimarin protects against free radical damage by increasing the production of antioxidant enzymes such superoxide dismutase, glutathione peroxidase, and catalase, which have all

been associated to diabetes. For this reason, it has been noted that silymarin can be used as a protector against nephropathies developing in diabetic patients (20). A study on nephrotoxicity caused by methotrexate in rats reports that silymaria balances oxidant-antioxidant status, regulates immunomodulatory functions, and inhibits inflammation, leading to a significant reduction in kidney damage (21). Research looking at the protective effects of cadmium poisoning in newborn rats' kidneys, cadmium reduces the overall volume of the kidney, medulla, and proximal and distal tubules, while increasing interstitial tissue (22).

2.3. Protective Effect of Silymarin on the Heart

Discovered to be an excellent protective agent against several kinds of poisoning produced by metals, environmental pollutants, oxidative agents, and drugs such as doxorubicin in a study against chemical-induced cardiotoxicity (23). In a research on animals with acrolein cardiotoxicity, silymarin was found to have protective benefits against the acrolein cardiotoxic site by lowering lipid peroxidation, renewing antioxidant enzyme activity, and avoiding apoptosis (24). They found that treating diabetic animals with silymarin can protect cardiomyocytes from apoptosis and regenerate pancreatic -cells in a study exploring the cytoprotective impact of silymarin against cardiomyocyte apoptosis induced by diabetes in diabetic rats (25). Nabavi et al. (2012), in a study examining the effects of sodium fluoride-induced toxicity and oxidative stress on rat heart tissues, reported that silymarin reduced lipid peroxidation, prevented superoxide dismutase, catalase activity and loss of glutathione level. Thus, they have reported that it had a positive effect on oxidative stress parameters (26).

2.4. Protective Effect of Silymarin on Testis

According to a study on its preventative effects on cadmium chloride-induced toxicity in mice, silymarin can significantly reverse the toxic effects of cadmium chloride and also reduce its negative effects on testicular histopathology, testosterone levels, oxidative stress indicators, and antioxidant defense enzymes (27). Silibinin was shown to maintain testicular tissues and spermatological parameters in a rat study of nickel sulfate-induced reproductive damage (28). In a study that looked at its protective effects on varicocele-induced testicular damage, as well as its effects on sperm parameters and antioxidant status, animals given silymarin were protected against testicular atrophy caused by

varicocele (29). A study looked at the radioprotective effects of silymarin on sperm parameters in mice (30).

2.5. Effect of Silymarin as an Anticancer Agent

The antioxidant properties of silymarin also serve as a protective agent in cancer. Therefore, it has been reported to show its cell-protective effect (31). It has been reported that silymarin inhibits benzoyl peroxide-induced skin cancer (32). Silymarin suppresses the multiplication of oral cancer cells and induces caspase-dependent apoptosis, according to a study studying its anti-cancer effectiveness and molecular target in human oral cancer in vitro and in vivo (33). According to Kim et al.(2019) silymarin treatment reduced the viability and migration of human gastric cancer cells in a dose-dependent manner, indicating that silymarin suppressed cell proliferation and tumor formation through triggering apoptosis. They believe there is hope for cancer treatment (34). Kim et al. (2019) discovered that after administering silymarin to human gastric cancer cells, the viability and migration of cells dropped dramatically. This study showed that silymarin could be a potential treatment option for gastric cancer as it suppresses cell growth and tumor development. (34). Velmurugan et al.(2008) found that silibinin greatly decreased the generation of aberrant crypt foci generated by Azoximethhane in vivo in a colorectal cancer investigation (35). Silibinin has been proven to suppress the formation of polyps in another mouse model (36). The effects of silymarin doses on the Slit-2/Robo-1 signaling pathway in hepatocellular carcinoma cells and Chemokine Receptor Type 4 (CXCR-4), which plays a role in the metastasis process, were investigated in a study (37).

According to a study that looked at the efficiency of silymarin gel in preventing radiodermatitis in breast cancer patients, silymarin considerably decreased the severity of radiodermatitis (38). Silymarin has the ability to decrease the survival, migration, and invasion of cervical cancer cells, according to a research investigating its potential efficacy against cervical cancer (39).

2.6. The Role of Silymarin in Alzheimer's Disease

Silymarin is well-known for its pharmacological effect in the neurological system, particularly in the treatment of Alzheimer's disease. Silymarin inhibits the precursor of APP (amyloid precursor protein), inhibits the polymerization of A, and raises the acetylcholine concentration in the nervous system by decreasing cholinesterase activity, according to research (40). In another study, Yaghmaei et al.(2014) they report that silymarin reduces Amiloid-β agregasyonu(Aβ)

by effectively suppressing the expression of APP in the rat brain leading to a decrease in the amount of APP (41).

In addition, it is noted that silymarin reduces Aβ plaque in mice with Alzheimer's disease and subsequently reduces memory deficits in the brains of mice (42). Exosomes generated from macrophages have been discovered to dramatically alleviate cognitive abnormalities in alzehimer mice in recent studies. Exosomes containing silibinin were utilized to reduce both Aβ aggregation and astrocyte activation in Alzheimer's model mice, and afterwards to alleviate cognitive impairment (43).

3. Conclusıon

As a result, it is known that silymarin and its main component silibine play an important role in various diseases. These diseases appear to cause significant damage to various organs and tissues. As a result of studies with silymarin, positive effects are seen on cytokines, antioxidants and some biochemical parameters. In this context, we believe that the mentioned miracle flavonoid can be an alternative to the agents currently used in the prevention/treatment of adverse effects of these diseases.

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ANIMAL THERAPY WITH NUTRACEUTICALS

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1.   Introduction

Nutraceuticals are products those are prepared using various foodstuffs and marketed in pills, powders, liquids or different pharmaceutical forms. Nutraceutical means those nutrition and pharmaceutical. Therefore, nutraceuticals are natural bioactive compounds that have a nutritional role as well as a supportive role when used with other drugs in the prevention, healing and treatment of diseases (1, 2). Thanks to these properties, they are reported to be new agents in the cure of various diseases such as diabetes, cancer, cardiovascular problems, atherosclerosis, and neurological disorders (3, 4, 5, 6, 7).

2.   Herbal Bioactive Components

Medicinal plants contain much active components such as alkaloids, terpenes glycosides, tannins and flavonoids which have developed curative or regulatory effects and are well-considered safe in long-term use (8). The active components of these plants can alleviate diarrhea by interacting with various cell types in the gastrointestinal system where contains epithelial and immune cells (9). In addition, essential oils, phenolic compounds, alkaloids, terpenes, glycoproteins, saponins, polysaccharides, tannins, mucus and many other substances in plants increase the phagocytic activity of macrophages, the number of B and T lymphocytes and the synthesis of interferon. These substances are mostly found in *echinacea, garlic, aloe vera, thyme* and *nettle* (10).

2.1. Anthraquinones

Anthraquinones consist of a number of compounds with multiple biological effects (11). It often has a laxative effect due to its stimulating effects on muscle contraction. Multiple plant species which have rich in anthraquinones include *Aloe vera*, watermelon, *Rumex crisp*, *Rheum palmatum* and *Senna*. Researchers reported that *aloe vera,* a hydroxyanthra, has the ability to limit cell growth in a variety of tumor lines, including lung carcinoma cells, hepatoma cells, and leukemia cells (12, 13).

2.2. Alkaloids

Alkaloids play important roles in the biological phases of microorganisms, plants and animals. It is a nitrogen-containing heterocyclic chemical compound which is used in pharmacological, medical and ecological areas. (14). Since alkaloids have an intense physiological effect on reaction with different levels of cellular bodies, they are widely used in the pharmaceutical field as therapeutic drugs (1). Alkaloids are solvable in organic solvents but highly toxic even in little volumes (15). Alkaloids obtained from plants are widely used in the drug areaindustry as antimalarial, anti-cancer and blood circulation (brain) promoting (vinkamine) agents (16).

2.3. Saponins

Multiple herbals containing saponins include, red pepper , ginseng and yucca (1). Saponins reduce Low density lipoprotein- cholesterol, serum cholesterol and glucose levels. It provides an increase in antioxidant, antifungal and neurotrophic activity (17).

2.4. Tannins

Tannins have many beneficial health properties such as anti- inflammatory, analgesic, antileishmanial, neuroprotective, immunomodulatory, antimicrobial, anti-lymphocytic, antidiarrheal and antihypertensive activities, and are also used in the treatment of ulcerative colitis (18). Tannin-containing legumes have been used as nutraceutical at livestock to maintain protection against gastrointestinal nematodes (19).

2.5. Essential Oils

Essential oils are obtained from buds, fruits, herbs, flowers, leaves, bark, seeds, roots, branches and wood parts (20). Essential oils includes significant levels of

terpenoids and have important activities againts to microorganisms (21). It has anticancer, antispasmodic and anti-inflammatory effects and has a good effect on the heart problems and circulatory system diseases (22). Apart from this, it has been used in tonics and medicines (fennel, marjoram and rosemary) that increase appetite, stimulate digestion and have a positive effect on absorption.

2.6. Carotenoids

Plants, bacteria, algae and fungi can synthesize carotenoids. However, animals cannot synthesize carotenoids and must metabolize them for using physiological functions through the diet (23).

2.7. Flavonoids

The medical effects of flavonoids include a protective role against coronary heart diseases, antiviral, antiallergic, anticholinesterase, anticancer and antioxidant activities (24). It plays role like nutraceutical and has multiple effects like anticancer, antioxidant, antidiabetic (25).

3. The Role of Nutraceuticals in Some Animal Diseases

For many years, scientists have sought to correlate phytochemical components and roles of them in health benefits. Studies show that the using of vegetables and reduces the danger of diseases of the all gastrointestinal systemes and inflammatory system (26). Minerals, vitamins, folic asit and other essential compounds have high important roles against diseases.

3.1. Nutraceuticals Used in Cat and Dog Diseases

In small animals, *Allium sativum and Calendula* officinalis are effective against *intestinal worms* for antiparasitic purposes; It has been reported that *Olea europaea* and *Artemisia annua* are effective against roundworms, and *Juglans nigra* is used against helminths.

　　Achillea millefolium, Rumex obtusifolius, and Juniperus communis as an antidiarrheal agent for gastrointestinal diseases of cats and dogs, *Aloe vera* as an anti-vomiting agent; *Elytrigia repens*, *Mentha piperita, Melissa officinalis* in cases of gastroenteritis; *Rumex crispus and Plantago ovata* in the treatment of constipation; It has been reported that *Ulmus fulva* is used in food poisoning (27). *Garlic*, along with its antimicrobial and other beneficial properties, has also been reported to be a useful herb for the holistic treatment of ear problems in pets, with antifungal activity against *Aspergillus fungus* (28).

3.2. Nutraceuticals Used in Ruminant Diseases

As an anthelmintic; Indian ginseng, *Withania Somnifera*, also known as poisonous gooseberry, has been reported to be effective on *Haemonchus concortus* in sheep (29).

It has been reported that abomasal helminths are rarely encountered in ruminants fed on gazal horn (*Lotus corniculatus*) and *white chicory* (*Cichorium intybus*) (30). It has been reported that grape pomace extract is effective against gastrointestinal nematodes (95% *Haemonchus concortus*, 5% *Trichostongylus sp.*) of sheep. In the study, it was reported that 0.30 mg/ml application inhibited hatching, 1.01 mg/ml application inhibited larval development and prevented larval migration 100% (31).

The farmacological use of herbals in the curve of diarrhea has recently become a popular practice (32). Immature fruits of *Papaver somniferum* (poppy), *Platanus orientalis* (poplar) berries, *Quercus coccifera* (gorse, pelite) *bark*, *Verbascum pycnostachium* (bear's ear, cow's ear) leaves, *Anarcardium occidentale, Psidium guajava, Myristica fraschinicalis, Myristica Coptischino-nicalis, and It is reported that Atractylodeslancea, Prunusmume and Poriacocos granule* mixtures play an effectively rol for treatment of ruminants' diarrhea (33). In addition, it has been determined that the use of thyme essential oil as a supplement in the treatment of calves with diarrhea, at a daily dose of 10 mg per 1 kg body weight, has beneficial effects (34).

It is stated that *Cassia fistula* fruit and *Punica granatum* peels show anti-*Escherichia coli* activity thanks to the phenols, flavonoids, anthraquinone, saponins, glycoside, steroids, and terpenoids components they contain (35).

It has been reported that plants which contains essential oils, tannins, flavonoids and saponins improve fermentation of rumen and increase nutrient usage in ruminants (36). Ayrle and friends reported that *Allium sativum (garlic), Mentha x piperita (mint) and Salvia officinalis (Sage), Camellia sinensis (tea), Matricaria recutita (German chamomile)* have an important potential in the treatment of digestive system diseases (37). Similarly, it has been reported that rumen functions are regulated by using gentian spp root, which is also called gentian and St. John's Wort, as an other way to the curve of lactic acidosis in sheep (38). In addition, it has been reported that garlic oil, anise oil, cinnamaldehyde, the main active ingredient of cinnamon, gentiana lutea (serpentine), thyme and clove plants can be used as an alternative to synthetic antimicrobial agents (39).

4.   Dosage in Nutraceuticals

The dosage of a nutraceutical agent is an important factor depending on the situation in which it is recommended to use. In terms of toxicology, nutraceuticals are generally considered safe at dietary doses (40). Ingesting of high doses of nutraceuticals can be toxic, due to the presence of substances that can cause genetic defects in target tissues. Herbal products contain health- promoting vitamins and natural biological substances, but ingesting of them extremely high can have toxic effectsç In addition, ingestion of specific type of polyphenol reduces the risk of cancer, but extremely intake of polyphenols can cause toxic cases. Herbals are commonly harmless but can have some toxicity effects when ingestion in high level amounts. In a study, it was noticed that extremely eating of *garlic juice* (4 ml/kg) or *garlic oil* (100 mg/kg)) may make unwanted effects, as weight loss, and kidney toxicity (41). In a similar study, it was stated that high doses of onion (500 mg/kg) could be problem in tissue and lung damage (42). However, it has been stated that daily eating of *garlic* (5-6 cloves), *onion* (50 g) g), *turmeric powder* (1 pinch) and *fenugreek seeds* (25-50 can prevent diabetes. Similarly, regular ingestion of curcuminoids (0.5 g) reduces blood lipid peroxide levels by up to 33%.

Mushrooms have some anti-nutritional elements such as hemagglutinins and phytates. Therefore, it is valuable to get the ideal dose of every food material. The weak immune system of a person depends on many factors. The most important of these factors are genetic predisposition and changes in lifestyle. Therefore, the responses of individuals to nutraceuticals may vary between persons.

5.   Conclusion

Nutraceuticals are an alternative source of natural medicine that both prevents and also cures many life-threatening diseases. It can be found more easily than prescription drugs. Because they are low cost and easy supply. However, studies on the effects of nutraceuticals against pathogenesis and disease activity are needed. In addition, the effect of bioactive components with other nutrient compounds and the effects of this interaction on the effectiveness ofbiological materials to act as drugs need to be investigated. Faster, more accurate and standardized clinical trials are required to enlarge the suitability of these yields in the global field.

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ANTIOXIDANT EFFECTS AND CLINICAL RESULTS OF ALPHA LIPOIC ACID USE

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Introduction

Lipoic acid was first defined in the 1930s and purified from liver tissue in the 1950s; its molecular structure is 1,2 dithiolene-3 pentanoic acid (1). Lipoic acid has a dithiolene-3 pentanoic acid ring structure and is an 8-carbon compound containing 2 sulfur atoms, and it can be synthesized in liver tissue as well as from octanoic acid and cysteine by various enzymatic reactions in mitochondria in plant and animal tissues (2). Lipoic acid can be transformed into oxidized and reduced forms by undergoing S-methylation and ß-oxidation inside the cell. While these oxidized and reduced forms show activity in the organism, the biologically active form is dihydrolipoic acid (DHLA), also called the reduced form. Lipoic acid is found in plant products such as spinach, tomatoes, and broccoli, as well as in animal foods such as heart, kidney and liver. Lipoic acid is also reported to be found in the structure of enzymes such as pyruvate dehydrogenase and alpha-ketoglutarat dehydrogenase, and to play a role in many metabolic events in the body, including ATP production.

1.   Biological Effects

Alph- lipoic acid has been found to be of potential use in the treatment of many diseases in various studies. Lipoic acid has been found to decrease the oxidized forms of other antioxidants, regulate nuclear factor kappa B (NFKB) and insulin signal pathways, decrease oxidative stress after exercise, decrease endothelial dysfunction, contribute to wound healing, and also decrease edema, inflammation, adhesion and fibrosis in damaged tissues (3)

2.   Antioxidant Effects

Alpha lipoic acid (ALA) contains a thiol group. It has high reducing properties through the dithiolane ring. When alpha-lipoic acid (a-la) (3,4) comes into contact with free radicals, the a-la molecule is oxidized and transformed into dihydrolipoic acid (DHLA). Lipoic acid acts as a cofactor in mitochondrial enzymes and occurs naturally (5). Lipoate, or its reduced form dihydrolipoate, reacts with reactive oxygen compounds such as hydroxyl, superoxide, and peroxyl radicals. Lipoate can function as a reducing regulator of proteins such as prolactin, myoglobin, and thioredoxin (6,7,8). Lipoic acid also plays a role in energy production and metabolism and is important in mitochondrial dehydrogenase reactions. Alpha lipoic acid has been shown to reduce fibrosis by stopping the PAI-1 inhibitor involved in fibrosis (9). Lipoic acid also protects membranes by interacting with glutathione, which regenerates vitamin E and vitamin C. Alpha lipoic acid, which has an anti-inflammatory effect, may indirectly affect the continuity of the cellular antioxidant defense by increasing the levels of other natural antioxidants such as tocopherol, glutathione (GSH) and ascorbic acid (10). Alpha lipoic acid (ALA) is an antioxidant substance found in foods like rice, peas, liver, eggs, tomatoes, and heart, and is naturally synthesized in the body. It has been shown that the administration of alpha lipoic acid may be beneficial in ischemia-reperfusion injury, diabetes, HIV activation, cataract development, nerve degeneration, and radiation damage. Alpha lipoic acid has been reported to have positive effects in various pathological conditions such as multiple sclerosis, diabetes, neuron degeneration, atherosclerosis, and joint diseases, due to its strong antioxidant structure (10,11)
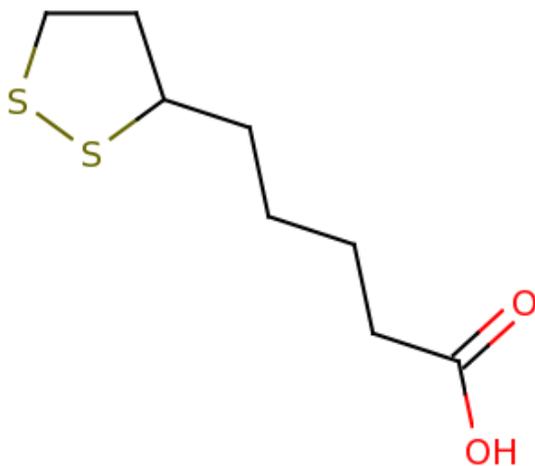
Figure 1. The chemical structure of alpha- lipoic acid

ALA has been shown to have positive effects on wound healing via its strong antioxidant properties and by decreasing signs such as inflammation and edema at the injury site (12). ALA is a powerful biological antioxidant and is available for oral and intraperitoneal use (10,13). ALA reaches peak concentration half and two hours following administration and is rapidly metabolized (13). It has been found to decrease oxidative stress by increasing the GSH level due to its strong antioxidant effect following both oral and intraperitoneal use, through a decreased MDA level in the tissues with adhesion in the study conducted by Özler et al. (13) and Diken Allahverdi et al. (3).

A significant decrease in edema, hyperemia, inflammation, fibrosis, and adhesion in the intestinal tissue was observed as a result of intraperitoneal ALA administration in the study performed by Diken Allahverdi et al. (3). ALA was administered during the intraoperative process in that study. The reason is that inflammation begins within minutes after surgical trauma, and the drug must reach an effective concentration at the time of injury (3).

Obstructive jaundice can develop due to various causes such as benign and malignant tumors of the bile duct, biliary surgery, and biliary pancreatitis. It results in a structural and functional disorder of the hepatobiliary system and can cause serious complications such as sepsis, renal and hepatic dysfunction, gastrointestinal hemorrhage, coagulopathy, cardiovascular dysfunction, and peripheral vasoconstriction. These complications are among the important problems of general surgery due to the mortality and morbidity they cause (14,15).

The endotoxemia that develops in obstructive jaundice triggers a pro-inflammatory process, leading to free oxygen radical production. The glutathione (GSH) enzyme system has an important role in decreasing oxidative damage and also has a preliminary defense role against oxidative stress, acting as an antioxidant. Glutathione is a very important mechanism in intracellular free radical detoxification and also lipid peroxidation prevention.

Glutathione plays a major role in the cellular defense against oxygen radicals. The liver MDA(Malondialdehid) levels provide an indication of lipid peroxidation due to free oxygen radicals in the tissues, and thus cell damage. MDA is the final product of lipid peroxidation and its levels increase following tissue damage caused by lipid peroxidation (16,17). The unique enzymatic control systems of catalase, superoxide dismutase (SOD), and GPX glutathione peroxidase provide protection against free oxygen radical tissue damage in the body, as increased activity of these enzymes contributes to decreased tissue damage (18).

Alpha lipoic acid plays a role in the reduction of tissue damage as an antioxidant, by increasing the activity of the relevant enzymes. M.H.Somi et al. have administered 25 mg/kg alpha lipoic acid after creating obstructive jaundice in rats and have then evaluated the AST(Aspartat aminotransferaz), ALT (Alanin aminotransferaz), GGT (Gama-glutamil transpeptidaz), bilirubin, MDA, SOD, and GPX(Glutatyon peroksidaz) levels. AST, ALT, GGT, and bilirubin levels were found to decrease in the obstructive jaundice group administered alpha lipoic acid compared to the control group. In addition, SOD and GPXlevels increased and the MDA level decreased in the alpha lipoic acid group compared to the control group (l9). Somi et al. have reported decreased histopathological damage in the liver and ileum with 25 mg/kg alpha lipoic acid when obstructive jaundice was created in rats (l9).

Hepatic ischemia and reperfusion damage were created in rats in the study conducted by Dulundu et al. (20). A decrease was found compared to the control group regarding the AST, ALT and MDA levels in the group given 100 mg/kg alpha lipoic acid, and an increase was found in the GSH level (20). Increased bilirubin levels and liver enzyme activities in obstructive jaundice are an indicators of liver damage, cholestasis, and hepatic dysfunction. A decrease in the polymorphonuclear leukocyte count and the levels of cytokines such as TNF alpha and IL1B in liver tissue has been reported by the same authors. Alpha lipoic acid has been found to decrease liver damage in an ischemia and reperfusion model (20)

In the Dünschede et al. study on 24 patients with portal triad ischemia and reperfusion damage caused by the Pringle maneuver, the alpha lipoic acid group's AST and ALT levels showed a statistically significant decrease compared to the control group when 600 mg alpha lipoic was administered 15 minutes after the maneuver (21). The authors also reported decreased hepatocyte apoptosis in the alpha lipoic acid group (21).

Rats with thioacetamide-induced liver cirrhosis were administered antioxidant substances such as curcumin, silybin-phytosome and alpha lipoic acid (200 mg/kg oral) in the Ali et al. study. The group receiving alpha lipoic acid had decreased AST, ALT, ALP(Alkalen Fosfataz), GGT and bilirubin levels, and this effect was more prominent when compared to the administration of other antioxidant substances (22). Ali et al. also administered alpha lipoic acid to rats with thioacetamide-induced liver cirrhosis and found it to inhibit collagen deposits and free oxygen radical production, decrease hepatocyte satellite cell function, and prevent liver cirrhosis progression (22).

Min et al. have reported decreased liver fibrosis in the group administered alpha lipoic acid compared to the control group in an obstructive jaundice model, and alpha lipoic acid appeared to stop plasminogen activator inhibitor activation in addition to decreasing liver fibrosis (23).

Shimaa et al. have administered alpha lipoic acid at doses of 20 mg/kg and 100 mg/kg for acetaminophen hepatotoxicity, and compared the protection provided after simultaneous administration of 20 mg/kg alpha lipoic acid and acetaminophen, and 1.5 hours after acetaminophen administration. While hepatotoxicity decreased in the low-dose alpha lipoic acid group, there was no such effect in the high-dose group. The reason has been postulated as the side effects of high doses of the drug, as they have been reported to decrease the protection provided, while low doses can protect the liver (24).

Free radical capture: Lipoic acid and dihydrolipoic acid (DHLA) are known to clear peroxynitrite, hydrogen peroxide, hypochloric acid-like reactive oxygen as well as nitrogen species, and are also known to support the antioxidant capacity of the cell by reinstating the formation of antioxidants such as vitamin E and ascorbate (Table 1) (25).

Table 1: Reactive oxygen and nitrogen species capturing properties of alpha lipoic acid and DHLA

Antioxidant Property	Alpha Lipoic Acid	DHLA
Hydrogen Peroxide	+++	+++
Monooxygen	+++	-----
Hydroxyl radical	+++	+++
Nitric oxide radical	+++	+++
Superoxide radical	-----	+++
Hypochlorous acid	+++	+++
Peroxynitrite	+++	+++
Peroxyl radical	-----	+++

Detoxification

Lipoic acid creates chelates with some pro-oxidant minerals, and it can also promote detoxification by neutralizing the pro-oxidant minerals in the organism.

Lipoic acid creates chelates mostly with Pb2+, Mn2+, Cu2+, and Zn2+, while dihydrolipoic acid creates chelates with Hg2+ and Fe3+ (21). Reactive oxygen species are known to be created in the organism following hydroperoxide breakdown, with the involvement of metals such as copper, iron, mercury and cadmium (21).

The Relationship of Alpha Lipoic Acid with Other Antioxidants

Glutathione, vitamin E, ascorbate, and coenzyme Q-like antioxidants become oxidized after they capture free radicals. Dihydrolipoic acid (DHLA) can similarly restore antioxidant properties by reducing oxidized antioxidants. Vitamin E is transformed into the tocopherol radical when it captures the peroxyl radical. Reduced glutathione and ubiquinol can form antioxidant networks by reducing these radicals. Dihydrolipoic acid (DHLA) can reduce various antioxidants and regenerate them through enzymes such as glutathione reductase. On the other hand, lipoic acid can reduce antioxidants in both hydrophilic and lipophilic environments (26).

The Relationship of Alpha Lipoic Acid with Diseases

Alpha lipoic acid has R and S forms. An effect of the R form on glucose transport has been reported and the R-enantiomer of alpha lipoic acid has been found to be effective in regulating and balancing glucose levels. The S form does not have an effect on regulating glucose levels. There is a consensus that alpha lipoic acid supplementation can regulate glucose levels in Type 1 and Type 2 diabetes (27). Oxidative stress is increased as a result of polyol pathway activation and the increase in glycosylated end products and reactive oxygen radicals in diabetic patients. This mechanism also plays an important role in the development of diabetic complications. Various studies have reported that lipoic acid can prevent these complications and regulate blood sugar, thanks to its antioxidant properties (28). Lipoic acid has also been reported to improve motor nerves in diabetic neuropathy and protect peripheral nerves from possible ischemia (29).

Lipoic acid supplementation has been reported to increase total antioxidant capacity without changing glutathione peroxidase activity, and also to improve memory problems and cognitive functions by reducing neurodegeneration in the hippocampal region by decreasing malonaldehyde levels. This is believed to be through the powerful antioxidant effect of alpha lipoic acid and the resultant decreased oxidative stress (30). It has also been reported in mouse models with

multiple sclerosis and encephalomyelitis that the substance stabilizes the blood-brain barrier, decreases inflammation, and may also have a preventive effect regarding oxidative stress by reducing reactive oxygen species (31).

Alpha lipoic acid can potentially be used after surgery to decrease the adhesions in the intestinal tissue as demonstrated in the intestinal adhesion model formed in rats by Diken Allahverdi et al., but this needs to be supported with future studies (3).

Improved biochemical values, decreased bile duct inflammation, decreased hepatocyte hydropic degeneration, and decreased liver necrosis and fibrosis have been found after alpha lipoic acid administration in rats with obstructive jaundice in the thesis work named "The effects of alpha lipoic acid on the liver in the obstructive jaundice model created in rats" conducted by Diken Allahverdi et al. This suggests that future studies may demonstrate the beneficial clinical use of alpha lipoic acid in patients with obstructive jaundice (32).

Conclusion

Alpha lipoic acid and its reduced form, dihydrolipoic acid, help decrease oxidative stress in the body due to their strong antioxidant properties. Alpha lipoic acid is a molecule with antioxidant characteristic. Lipoic acid is synthesized by lipoic acid synthase found in mitochondria in physiological systems . Alpha lipoic acid derivatives react with oxygen radicals and remove them from the environment . Alpha lipoic acid has also been shown to decrease fibrosis by stopping the PAI-1 inhibitor that plays a role in the fibrosis process. This antioxidant effect is achieved with the help of mechanisms such as creating chelates with metals, increasing the reusability of other antioxidants, capturing free radicals, and repairing oxidative damage. Alpha lipoic acid is a powerful antioxidant, and can prevent the progression of neurological disorders such as diabetes, diabetic neuropathy, and Alzheimer's disease.Studies have also shown that it can prevent intra-abdominal adhesions after surgery and has antiadhesive, anti-inflammatory, and fibrosis prevention properties, especially in studies on the liver. We believe that Alpha Lipoic Acid can be used in the clinic after surgery and in cases with obstructive jaundice in future studies.

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