TÜRKİYE CUMHURİYETİ'NİN YÜZÜNCÜ YILI

# Current Overview of Herbal Antioxidants and Research

**Editör**

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Current Overview of Herbal Antioxidants and Research

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# PREFACE

Black cumin seed has been used for thousands of years for the treatment of diseases, flavor and aroma. Today, it is widely used in pharmacy, food industry and cosmetics due to its antioxidant, antiviral and antibacterial effects. The biological active components, physiological and clinical effects of black cumin seed, especially antioxidant, anti-inflammatory, antidiabetic, antihistamine, anticonvulsant, anti-tumoural and antihypertensive mechanisms of action are discussed in detail.

Antioxidants play a role in detoxification of free radicals that develop during metabolic reactions and prevent harmful effects on the organism. Plant-derived antioxidant compounds have long been investigated for their beneficial effects in disease prevention. The effects of sanggenon C as a new, effective and safe antioxidant as a flavonoid compound obtained from the root bark of white mulberry have attracted attention in complementary medicine and pharmaceutical research in recent years. In this context, the antioxidant, antitumor, antiviral and anti-inflammatory effects of sanggenon C have been extensively investigated.

Basic behavioral, biochemical and physiological information about laboratory animals, especially rats, mice, rabbits, guinea pigs, hamsters have an important role in biomedical researches of all researchers. Knowing the anatomy, physiology, hematological and biochemical parameters of laboratory animals will enable researchers to know the animal they are working on, to ensure animal welfare, to understand the findings obtained, to care and feed animals under appropriate conditions, to prevent diseases and thus to carry out studies correctly and successfully.

This book is presented to its readers as a basic resource containing up-to-date information that all researchers working in the field of health can benefit from.

**Editor**

Prof. Dr. Başak Hanedan

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# CHAPTER I

## Nıgella Satıva: Bıoactıve Components, Physıologıcal And Clınıcal Effects

**Recep ASLAN[1]**
**Selinay BORAZAN[2]**

Medicinal and aromatic plants have been used for thousands of years, not only for the treatment of many diseases, but also for flavor and aroma purposes. Today, medicinal aromatic plants are widely used in areas such as pharmacy, food industry and cosmetics due to the broad-spectrum properties of the phytochemicals they contain with effects such as antioxidant, antiviral and antibacterial (Gün, 2012).

Black cumin seeds, which have an important place as a traditional medicine and food spice in Muslim societies, have been

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blessed with the teaching that "There is no disease except death for which there is no cure in black cumin" (Buhari, Tıp); this teaching has led to the acceptance of black cumin as a medicinal plant of all times; black cumin seeds are considered as "fertile seeds" due to their therapeutic properties for many diseases. Black cumin was also recognized in other societies and physicians such as Hippocrates and Dioscorides used black cumin for therapeutic purposes. Black cumin seeds found in some sarcophagus finds are accepted as data showing the relationship of black cumin with ancient societies. For all these reasons, it has also attracted the attention of modern medicine, and its thymoquinones, other bioactive substances and phytochemicals have been the subject of research and preventive-therapeutic applications. Black cumin seeds and oil are still widely used in folkloric medicine and complementary medicine applications today, although they have not yet completed drug phase trials and have not become pharmaceutical formulations. Despite all these, the fact that it has not been discussed during the pandemic process and that awareness studies have not been carried out on its protective effects against the Covid-19 pandemic should be seen as a deficiency (Ragaa, 2010).

## Nigella Sativa

Nigella sativa L. is a member of the buttercup family (Ranunculaceae) and is cultivated in many places in Anatolia, especially in Afyonkarahisar, Burdur, Isparta and Konya. It is cultivated globally mostly in Europe, Southwest Asia and North Africa in clayey and hot soils that are not excessively sandy, rich in nutrients. Nigella Sativa L., called "black seed" by the Arabs and "black cumin" by the British, is known as "black cumin seed" in our country. Black cumin seed fruits in the form of flavored, bitter black seeds can be added to foodstuffs, herbal teas, and coffee mixtures. Thousands of scientific studies have been published on black cumin, which is used in sectors such as food, medicine, cosmetics, and paint, in addition to medicine. However, as a medicinal and aromatic plant, specific and safe usage forms, effective amounts, and dosage information are still not established. Nigella sativa, one of the three common species of the Nigella genus, is also popularly called black sesame or black sesame. Species of the buttercup family (Ranunculaceae) include wild black cumin (Nigella arvensis), pinwheel nigella (Nigella ciliaris), Damascus nigella (Nigella damascena), Spanish nigella (Nigella hispanica), Nigella integrifolia, Nigella nigellastrum, yellow nigella (Nigella orientalis), Nigella oxpetala, common nigella (Nigella sativa) and Nigella segetalis. Although the seed in the capsule contains highly effective essential oils and phytochemicals, it is not a very oily seed. Important phytochemicals include thymoquinone, dithymoquinone and other essential oils, fixed oils, and proteins, melanthin, nigellin, saponins and tannins. Black cumin seed, which is also rich in vitamins and minerals, is an important source of vitamins A, H, C, B1, B2 and B6, folic acid and niacin (Gün, 2012).

Black cumin is an annual herb that can adapt to a variety of climates and grows in almost every climate. It is a 20-30 cm tall herbaceous plant, blooming in June-July with yellow, white, pink, pale blue, purple and green flowers, fragrant and usually light blue in color. It usually grows spontaneously along roadsides and in crop fields. The delicate flowers of black cumin, which have erect stems and lance-shaped leaves, usually consist of 8 petals. The black fruits are abundantly seeded and capsule-shaped, consisting of three to seven united follicles, each containing multiple seeds. Characterized by their black color, these seeds are flat, trifacial and funnel-shaped, 0.2 cm long and 0.1 cm wide (Bulca, 2014).

Different species of black cumin are grown commercially. Although it is a plant with about 2000 different species, Nigella sativa, Nigella damascena and Nigella arvensi species are the most widely known. In our country, it is widely cultivated in Afyonkarahisar, Isparta, Burdur, Konya, Samsun, and many other regions. In recent years, interest in black cumin cultivation has increased in Thrace and Aegean regions. The only commercialized species of the plant is Nigella sativa L. Nigella grows in Turkey, southern European countries, India, and parts of Africa with fertile soils. As a generalization, it can grow as a herbaceous plant on land and in water in biomes other than tropical regions in the Northern Hemisphere. However, areas with plenty of sunshine and mild climates are the most fertile environments for black cumin. In black cumin cultivation, special attention should be paid to growing the plant on sandy and slightly clayey soils, and the plant should be watered regularly. Black cumin is sown in spring and as early as possible. Depending on the type of soil it is grown in, it may require fertilizer supplementation, in which case higher yields are obtained. Black cumin, which is grown from seed, sheds its seeds at the end of each year and can grow again where it was. After the capsules of the black cumin plant burst, the plant is harvested. The chemical composition in Table 1 is directly affected by factors such as harvest season, type, climate, soil, and irrigation (Correa et all., 1986; Sultan et all., 2009; Ayhan, 2012; Gharby et all., 2015).

*Table 1: Main constituents of black cumin (El Yassir 1992)*

| **black cumin (ingredient)** | **amount (%)** |
|---|---|
| water | 7 |
| protein | 23 |
| fat | 39 |
| starch | 15 |
| crude fiber | 5,4 |
| dietary fiber | 16 |
| ash | 4,3 |

## Bioactive Phytochemicals of Black Cumin

Thymoquinone, thymohydroquinone and dithymoquinone are the first bioactive chemicals that come to mind among the bioactive chemicals effective in the formation of antioxidant, anti-inflammatory, antibacterial, antitumoral and immune stimulant effects of black cumin. It is suggested that the main active nutraceutical substance is thymoquinone. It has been reported that thymoquinone in the quinone structure with a phenol ring has a primary role in the biological activities of black cumin seeds, so the thymoquinone content in black cumin seeds should not be below

18%. Many scientific studies have reported that thymol, limonene, carvacrol, p-cymene, alpha and beta pinene, 4-terpineol, longifolene and t-anethol benzene were detected in black cumin seeds in addition to thymoquinone. Black cumin extract amino acids can be listed as follows according to their percentages: Glutamic acid 22.40%, Aspartic acid 10.05%, Arginine 9.18%, Lysin 6.92%, Glycine 6.86%, Pyrroline 6.07%, Valine 5.10%, Alanine 4.21%, Phenylalanine 4.00%, Isolysin 3.00%, Isolysin 3.00%, Phenylalanine 4.00%. 00%, Isoleucine 3.98%, Threonine 3.95%, Lysine 3.91%, Serine 3.80%; Tyrosine 3.35%, Histidine 2.83%, Methionine 1.45%, Cystine 1.17%, Tryptophan 0.77% (9). Black cumin seeds are rich in fatty acids, especially oleic, linolenic, linoleic, arachidonic, palmitoleic and stearic acids; they are also a strong source of carotene, flavonoids, indazole alkaloids, saponins, cardiac glycosides and iron, It has been shown that black cumin is rich in phosphorus, calcium, potassium, zinc and selenium (6-9%), and its seeds contain carbohydrate (35%), fat (omega 3, omega 6), vitamins (B1, B2, B3, B6 and folic acid) and essential amino acids. The fact that black cumin contains nigellicin, nigellimin and nigellidin, which are rare types of indazole alkaloids, makes it an even more valuable seed (Ayhan, 2012; Gharby, 2015).

It has been reported that factors such as the climate of the region where it is grown, the type of black cumin, and the time of sowing and harvesting affect the chemical content of the plant, although not characteristically. Black cumin seeds contain compounds such as fixed oil, essential oil, protein, fiber, organic acid, resin, carbohydrate, vitamin, mineral, alkaloid, tannin, saponin, amino acid, ascorbic acid, thiamine, niacin, pyridoxine, and folic acid. Seeds have also been shown to be rich in calcium, potassium, phosphorus, iron, magnesium, selenium, zinc minerals and vitamins A, C and B group vitamin. Black cumin fixed oils contain unsaturated fatty acids such as oleic acid, linoleic acid, eicosadienoic acid, eicosadienoic acid, arachidonic acid, linolenic acid, and saturated fatty acids such as myristic acid, palmitic acid, stearic acid; essential oils contain components such as nigellone, carvacrol, p-cymene, alpha and beta pinene, d-limonene, thymoquinone, dithymoquinone, thymohydroquinone and thymol. As in all herbal and medicinal plants, essential oils are of primary importance in black cumin. The biologically active compounds in black cumin essential oils are thymoquinone, dithymoquinone, thymohydroquinone and thymol. Thymoquinone, the most active compound of black cumin seed, is almost identified with this plant and contains a phenol ring and has a quinone structure (Güzelsoy et all., 2018; Yalçın, 2019; Gün, 2012; Asal et all., 2018).

## Nigella Sativa Essential Oils

Vegetable essential oils are natural products obtained from leaves, fruits, bark, or non-woody root parts of plants. They are liquid at room temperature, easily crystallizing, colorless or light yellow, volatile and have a strong odor. Their fragrance has led them to be called "essences" and "ethereal oils". They are defined as oils because they do not mix with water and are normally different from fixed oils. Today, about 2600 plant essential oils have been recorded and most of them are hydrocarbons, alcohols, esters, and aldehydes. Very few of them consist of paraffin and wax mixtures (Bulca, 2014). Essential oils such as thymoquinone, dihydrothymoquinone, carvacrol, p-cymene, alpha and beta pinene, alpha tujen and t-anethol make black cumin a qualified essential oil source. These oils are important antioxidants directly involved in the processes of suppression of oxidation and peroxylation in foods, prevention, and repair of cell damage due to free radical-induced oxidation-peroxidation reactions in human and animal organisms (Cheikh-Rouhou, 2007). The fact that the antimicrobial, antifungal, anti-inflammatory, anticarcinogenic, antidiabetic, antidiabetic and antioxidant properties of black cumin seed essential oils have been scientifically determined has increased the use of these oils not only as flavoring in products such as pastes, bread, cakes and other bakery products, cheese, and pickles, but also as protective and supportive medicinal plants (Vardar, 2008). Its functionality is also utilized in cosmetics and dietary supplements as well as in coffee, tea, and some salads. Antioxidant phytochemicals protect the quality of foodstuffs and increase the safety and shelf life of foods by protecting them against natural oxidation and peroxidation processes, provided that appropriate and high-quality raw materials, production techniques, packaging and storage methods and appropriate varieties are used in safe quantities. In vitro studies suggest that black cumin essential oil has a higher antioxidant effect than fixed oil, which is due to its polyunsaturated fatty acids. It was found that black cumin oil contained carotenoids and tocopherol at the level of 450 mg/kg of oil, and the seed contained 201.3 mg/kg of seed thymoquinone. Since the findings show that the extraction technique is directly effective on the composition and quality of black cumin oil and that the oxidation of black cumin oil produced by cold pressing technique is more sensitive than solvent extraction method, it is recommended to prefer oils produced by cold pressing technique (Gün, 2012; Bulca, 2014).

## Thymoquinone

Thymoquinone, the main phenolic component with antioxidant properties in Nigella sativa L. seed, has been shown to have anti-inflammatory, antimicrobial and anticancer effects. In cases with tissue damage due to oxidative stress, thymoquinone is also known to repair oxidative damage and protect against damaging effects. Black cumin oil and thymoquinone have protective effects against DNA damage, collect free radicals in the environment and are powerful antioxidants. Studies examining black cumin seeds in terms of antioxidant activity have found better activity than synthetic antioxidants. It has been reported that shoots and roots as well as seeds of black cumin plant are effective as natural antioxidants and show a strong protection against free radical-induced organoleptic deterioration. Despite all these data, there are also studies suggesting that thymoquinone increases reactive oxygen species and oxidative stress because of biotransformation after entering the cell and causes genomic damage in liver cells (Ali and Blunden, 2003). However, the metabolic mechanisms of action of thymoquinone have still not been concretized and its usability as a drug has not been reached (Vardar, 2008). Thymoquinone is the center of interest of many current studies due to its anticancer properties. Mechanisms related to its cytotoxicity and genotoxicity are being addressed in this respect. The most important effect of thymoquinone is that while it does not show cytotoxicity in healthy cells, it causes a strong cytotoxic effect in tumor cells (Darakhshan et all., 2015). However, most studies report that thymoquinone repairs oxidative stress-induced tissue damages. These publications also note the anticancer, anti-inflammatory, analgesic, hypoglycemic and immunostimulant effects of thymoquinone. It has been established that the main component of black cumin seeds is thymoquinone, this phenolic compound constitutes the most active pharmacologically active ingredient of black cumin seeds. There are quite a few studies showing thymoquinone, an essential oil, as a bioactive molecule with analgesic, antipyretic, antimicrobial and antineoplastic activity. It is suggested that thymoquinone regulates blood lipids, decreases plasma cholesterol, glucose, and triglyceride ratios, decreases blood pressure, and accelerates respiration (Güllü Bacak and Avcı, 2013). In conclusion, thymoquinone has been shown to have many therapeutic effects, especially anti-inflammatory, antimicrobial, and anticancer effects, and it has been observed that toxic effects may occur only at extremely high doses. Therefore, thymoquinone, with its high bioactive properties and very low systemic toxicity, should attract the attention of modern medicine and the pharmaceutical industry. Because these findings may be a promising alternative for therapeutic efficacy and successful outcomes that cannot be achieved with classical conventional therapeutics and drugs. This efficacy can be explained by the fact that thymoquinone transforms many molecular targets. Despite this broad spectrum of effects, the effects of thymoquinone on metabolic pathways remain to be understood (Gün, 2012).

## Nigella Sativa: Physiological and Clinical Effects

In traditional medicine, black cumin has been recognized and used for centuries as an important source of healing for many diseases by Muslim peoples in Asia, the Middle East, Africa, and Europe. Despite this, the therapeutic efficacy of black cumin oil and its extracts needs to be demonstrated by experimental studies. Studies conducted for this purpose suggest that black cumin oil and its extracts show antimicrobial effect in bacteria that have developed multiple antibiotic resistance and that this effect is mediated by thymohydroquinone and melanin, thymoquinone. Black cumin (Nigella sativa) inhibited the growth of bacteria such as Brucella abortus, Corynebacterium pseudotuberculosis, Corynebacterium renale, Pasteurella multocida, E. coli, Trueperella pyogenes, Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes. Studies on the effects on blood lipid profile report that black cumin seed oils reduce serum triglyceride and LDL cholesterol levels and support HDL cholesterol production. It is understood from the studies that black cumin can be used in the fight against diabetes, which is among the top three globally prevalent health risks, and that it is suggested to show antidiabetic effect by supporting regeneration in pancreatic islets of Langerhans. In epilepsy cases, the antiepileptic drug valproate (valproic acid) and black cumin oil were compared clinically, and it was reported that the anticonvulsant effect of black cumin oil in the epilepsy process was better than valproic acid. Antitumoral effects of black cumin active components are also remarkable. The suppressive effect of thymoquinone on cancer formation in cancers such as brain tumors, breast and ovarian adenocarcinoma, prostate cancer, neoplastic keratinocytes, gastric carcinoma, colorectal cancer, osteosarcoma, fibrosarcoma, lung carcinoma, leukemia has been reported in the literature, black cumin seeds and oil have been shown to increase T cells by 30-55% by stimulating the immune system, and black cumin seeds added to the diet have been shown to be effective in relieving fatigue and symptoms. Although it is a powerful immunostimulant, black cumin is also known to relieve and improve hypersensitivity symptoms in chronic allergic conditions such as asthma and rhinitis. Black cumin is also recommended as an antioxidant, black cumin seeds are effective in improving oxidative damage, significantly lowering malondialdehyde, an indicator of oxidative stress, and restoring the levels of glutathione transferase, peroxidase and reductase, antioxidant enzymes in the glutathione cycle. These findings suggest that black cumin seed is a powerful, rapid, and versatile oxidative stress reliever as a powerful multifunctional antioxidant (İlhan et all., 2005; Jamal et all., 2013; Namjoo et all., 2013; Kamil, 2013; Asal et all., 2018).

## Nigella Sativa in Folkloric Medicine

Although elements brought from Central Asia, the shared beliefs of ancient tribes, beliefs, and philosophies such as Christianity and Mazdeism are influential in the information about black cumin, known as the "fertile seed" in Anatolian folk medicine, the real importance and use is due to the data belonging to Islam (Gün 2012, Dündar 2015). Due to the hadiths on the theme of "Keep using this black cumin herb, because it has a cure for every disease except death" (Buhari, Tıp), black cumin herb has been given a special place in the minds of the people, a high level of curiosity, love and admiration has been created, and black cumin seeds and oil have been included in the medical practices of Islamic societies both for fitness purposes and in the treatment of different diseases. For this reason, black cumin seeds and oil are most widely used in geographical areas where Islam has been adopted and are preferred by Muslim populations in Europe and America, as well as in Central Asia, Anatolia, the Middle East and the Caucasus regions. The seeds, whole or ground, are added to breads, pastries and salads for healing purposes, and cold-pressed oil is also utilized.

Islam states that the root of many diseases lies in mistakes in thinking, beliefs, and lifestyle, and that most diseases are caused by "stress". Stress is caused by the ideas and interpretations one puts forward or the ideas and interpretations one encounters. In Islamic belief, if the individual aligns his/her interpretations, ideas, criticisms, and acceptances with the belief in "fate", there will be no event or factor that will cause him/her stress, and stress and stress-related diseases will be off the agenda. The biggest obstacle to a correct view of the system, life and destiny is the pathological use of the sense of "I", which in psychiatry is referred to as personality problems, identity disorder problems. In Islam, that is, in Tawhid thought, the "healing" in a plant, an object or a practice is associated with the knowledge, command and permission attached to the active substance in that object. For example, for the phytochemicals and bioactive substances in a plant to be effective, they must be authorized. Because in Islamic belief, there are no inanimate, insentient, unconscious objects. The following medical anecdote describing this approach is a case study of this thought and believed mechanism. It is narrated that when Hazrat Moses (as) used an herb that was taught to him one day when he had a toothache and the pain did not go away, he was told, "O Moses, the effect is not in the herb, but in the command given to it". Although this approach is taken more into consideration especially in Sufi culture, in Islamic belief, objects and their effects are viewed on this basis, that is, in fact, the effect and communication are based on the meanings they contain. When approached from this perspective, it will be easier to understand the hadith recommendations to recite certain surahs in certain diseases. Let us give an example of a well-known practice. Hadiths advise that "Fatiha is the Surah of healing". For this reason, Muslims use Surah Fatiha as a remedy for physical illnesses, diseases of the heart tissue and especially psychological illnesses and chaos due to "I-ness" and personality disorders. This is also how Islamic societies view black cumin seeds and oil, which have become the medicinal remedies of choice for almost everything in Muslim lands because they are based on the platform of faith in the knowledge reported in the hadith and the acceptance of what has been reported with foresight. Black cumin seeds and oil are used for many health problems such as asthma, cough, bronchitis, rheumatism, dyspepsia, diarrhea, dysentery, fever, aches, pains, gas and bloating, jaundice, paralysis, hemorrhoids, cancers, etc., based on the knowledge of its healing properties given in the hadith. Complementary medicine authorities, who do not deny religious recommendations but also look for evidence, use black cumin seeds and oil in their practices in line with the findings of scientific studies with black cumin active ingredients, predicting that black cumin seeds and oil may be beneficial in cardiovascular system, respiratory, digestive, and excretory system diseases, immune-related problems, liver damage and inflammation. Alternative and complementary medicine uses black seed oil to suppress depression and stress, strengthen immunity in diseases such as cancer, metabolic regulation, regulate blood pressure as well as fatigue and insomnia, wound healing, regulate intestinal peristalsis, relieve constipation, hemorrhoids, fight intestinal parasites, relieve rheumatoid arthritis and joint pain, relieve nausea, facilitate digestion, increase the amount and duration of milk production in puerperal, fight headaches, strengthening the cardiovascular system, preventing the formation of renal stones, opening the breath, relieving flu and sinusitis symptoms, suppressing the development of diabetes and its complications, regulating cholesterol levels, supporting cell proliferation and sexual function, It is used in many cases such as combating ear infections, treatment of asthma and other allergic cases, drainage of congested concha and sinuses, relief of muscle pain with local external application, hormonal regulation, fertilization, detoxification and appetite control (Gün, 2012; Dündar, 2016; Güzelsoy et all., 2018; Yalçın, 2019;).

**Nigella Sativa: From Folkloric to Conventional Medicine**

This is a continuation of the first article on the general properties and bioactive chemical content of black cumin seed, a medicinal aromatic plant that is trusted and widely used for multiple purposes, especially in the Mediterranean basin, Far Asia, and Muslim societies. Although conventional medical practices have made significant progress thanks to rapidly developing technology and its application to medicine, alternative medical orientations and practices attract intense interest in the sociological fields of all beliefs and cultures on a global scale and continue their vibrant and effective existence as a field frequently consulted by physicians and medical support recipients. Black cumin seed, one of the most important plants used in the treatment of diseases and health protection for thousands of years, is a phytotherapy element that is used today with its extracts, oils and essential oils obtained more easily and safely. Rising values such as Pharmacoeconomics, individualization, freedom of opinion and belief, and democracy support this process. As an indicator of this, Complementary Medicine Departments have been established in medical faculties in many countries, as in our country, and traditional medical practices have become feasible methods in hospitals, if they are as evidence-based as possible. Since their risk levels are quite low and they are believed to have healing properties, plants are considered within the scope of "phytotherapy" in scientific medical platforms as well as in folk practices. In addition to local plants that stand out in different societies, many plants that stand out according to the beliefs and cultures of societies and whose healing effects are utilized have been harvested, collected from nature, and used commercially or folklorically for centuries in acute and chronic diseases, as well as in preventive medicine). This study aims to pave the way for a scientific reorientation by reviewing the studies on the different medicinal effects of black cumin, which has a very important medicinal place in Islamic societies, under basic headings, and to initiate scientific studies on black cumin that will continue until it is transformed into pharmaceutical form. Verses and hadiths, which are accepted as data with the certainty of laboratory findings or even more proven for Islamic societies, directly affect the lives of individuals raised in this culture (Gün, 2012; Aslan, 2016; Yalçın, 2019).

Historical documents show that among the plants used for therapeutic purposes, black cumin has been known for thousands of years with its benefits as a remarkably important plant. Twelve different species of the Nigella genus of the Ranunculaceae (buttercup family) are widely known and cultivated agriculturally.

Among these species, "Nigella Sativa, Nigella Arvansi, Nigella Damascena" is widely used in folk medicine and as spices. Nigella Sativa is popularly known as "black cumin" and is widely cultivated. Due to the favorable climatic conditions in our country, it can be cultivated in almost every region, especially in Konya, Afyonkarahisar, Burdur, Isparta, Kütahya and Çukurova regions. Depending on the country or region where it is grown, the plant is called black cumin in England, black cumin seed in America, black grain, and black seed in Arab societies. It is a plant that blooms in June-July and attracts attention with its blue, white, and green colored fragrant flowers (Bulca, 2014; Asal et all, 2018; Yalçın, 2019)

## Nigella Sativa As An Antioxidant

Reduction and oxidation events are in a state of equilibrium in the organism. Free radicals, which have an unpaired electron and want to pair this electron, tend to participate in continuous reactions in redox events due to their unpaired electrons. "Antioxidant" substances that can prevent or delay the oxidation of a biochemical substance in the organism or end the process by breaking the oxidation chain protect the oxidation-reduction balance in the organism by suppressing the oxidation ability and reactions of atoms and molecules with free radical properties). When atoms and molecules with free radical properties increase in the environment, problems that may extend to cellular damage may occur depending on the reactions of these products. It is indisputably accepted that free radical-producing reactions and oxidative stress caused by these reaction products play a role in the pathogenesis of many diseases such as atherosclerosis, diabetes, hypertension, and cancers in the organism. This picture, which is frequently seen in the inadequacy of endogenous and exogenous antioxidant defense elements, is known as oxidative stress. Oxidative stress constitutes an important infrastructure for diseases such as atherosclerosis, also known as arteriosclerosis, diabetes mellitus, hypertension, and cancer. Thymoquinone, a characteristic bioactive component of black cumin seed and an antioxidant, attracts attention due to its ability to reduce free radicals in the environment, prevent lipid peroxidation, prevent, and repair tissue damage, and show protective effects against DNA damage and organoleptic deterioration. In vivo studies have shown that black cumin oil and its active ingredient thymoquinone reduce lipid peroxidation and thus may be a therapeutically effective antioxidant in diseases such as atherosclerosis, cancer, and rheumatism. Some studies have suggested that high levels of carotenoids, tocopherol and thymoquinone in black cumin oil delay the spoilage processes of foods by inhibiting lipid oxidation and may be a natural alternative to synthetic antioxidants used in food preservation; it has also been noted that seeds of black cumin plant have a stronger antioxidant effect than black cumin oil as an effective antioxidant. It has been shown that the antioxidant activity of black cumin occurs by preventing liposomal lipid peroxidation of both black cumin fixed oil and thymoquinone, one of its phenolic components. Black cumin oil has been reported to show antioxidant activity by scavenging 2,2-diphenyl-1-picrylhydrazyl and hydroxyl radicals. Researchers have suggested that bioactive components in black cumin oil such as thymoquinone, carvacrol, 4-terpineol, t-ethanol and quercetin are effective in suppressing the lipid peroxidation process and removing its products (İlhan et all., 2005; Bulca, 2014; Aslan, 2016; Yalçın, 2019; Burtis and Bucar, 2000).

## Nigella Sativa and Inflammatory Process

Today's medicine argues that the profile of disease has changed, that the profile of chronic diseases such as diabetes, rheumatism, hypertension, Parkinson's, Alzheimer's, cancers, and obesity is no longer microbial diseases, but rather chronic diseases such as diabetes, rheumatism, hypertension, Parkinson's, Alzheimer's, cancers, and obesity, and that the main reason for the increase in these chronic diseases is the phenomenon of inflammation. For this reason, much research has focused on how the processes of chronic inflammation can be prevented. Today, when chronic inflammation or chronic inflammation-based diseases have become an important mortality factor rather than a global risk, how this problem can be solved, how it can be treated or how it can be prevented is being discussed. Anti-inflammatory effects of thymoquinone, one of the bioactive components of black cumin seed, have been shown in many studies. The course of inflammation, which is a defense and repair process, is regulated by two enzymes. These enzymatic pathways are cyclooxygenase and lipoxygenase pathways. Prostaglandins are synthesized in the cyclooxygenase pathway and leukotrienes are produced in the lipoxygenase pathway. Thymoquinone exerts its anti-inflammatory effect by inhibiting both cyclooxygenase and lipoxygenase pathways. In this way, it suppresses the inflammation process by inhibiting thromboxane B2 and eicosanoid production (Güllü Bacak and Avcı, 2013; Müftüoğlu, 2016).

The picture of hyperinsulinemia (hyperinsulinemia), which is encountered in chronic inflammation processes, means insulin overload of the body. Although insulin is one of the essential and vital hormones for human and animal organisms, insulin overload is not a desired physiological picture in good medicine. Just as hypoglycemia due to its deprivation is a serious problem, an organism and physiological process with high insulin levels is a serious problem. One of the known side effects is that excess insulin leads to excessive accumulation and storage of fat in tissues. In addition, high insulin levels lead to a lifestyle characterized by chronic inflammation and obesity, as they cause food indulgence, excessive appetite and excessive eating and drinking behavior. High insulin concentration promotes chronic inflammation by accelerating the formation of adipocytes, known as fat cells, and increasing the production of cytokines, substances that trigger the inflammatory process leading to inflammation. Different types of inflammatory cytokines, also known as adipokines, such as ILB-1, ILB-6, TNF-alpha, are known to cause both insulin resistance and chronic inflammation in tissues. While high insulin accompanying chronic inflammation triggers insulin resistance, hidden diabetes, diabetes, weight gain due to hyperinsulinemia, belly, and obesity, it also paves the way for diseases related to chronic inflammation in different tissues. For this reason, diabetes is also recognized as an inflammatory disease. For the same reason, diabetic patients suffer from rheumatic problems, muscle weakness, tendon calcifications and carpal tunnel syndrome. The insulin resistance, hidden or overt sugar picture seen in Alzheimer's patients with widespread inflammatory picture and degeneration in neurons also points to this interaction. The anti-inflammatory effect of black cumin seeds, oil and extracts has been attributed to the thymoquinone it contains; it has been shown that thymoquinone and its derivatives can inhibit inflammation via cyclooxygenase (COX) and lipoxygenase (LO) pathways. The suppressive effect of thymoquinone on anti-inflammatory processes is specially produced by these two enzymes; the inflammatory COX and LO pathways are inhibited by synthesizing prostaglandins in the COX pathway and by leukotrienes produced in the LO pathway; the production and release of thromboxane B2 and eicosanoids are inhibited (Müftüoğlu, 2016; Yalçın, 2019).

## Antidiabetic Effect of Nigella Sativa

Diabetes is a metabolic and inflammatory disease characterized by an increase in plasma glucose levels due to insufficient insulin synthesis or insulin resistance. When black cumin and thymoquinone were administered to diabetic mice for four weeks, plasma glucose levels decreased significantly, and this result was thought to be due to thymoquinone-liver interaction. It was reported that thymoquinone suppressed glucose production from liver glycogen in diabetic mice, increased insulin release from the pancreas, and did not cause any side effect based on deprivation or excess in terms of any substance or process in the organism during this whole process (Asal et all., 2018; Yalçın, 2019).

## Immunity and Nigella Sativa

Immunity is an indicator of the organized defense system, and it develops defense against disease agents, pathogens, tumor cells and foreign proteins in the organism and tries to destroy and neutralize them. Macrophages, natural killer cells, granulated and non-granulated leukocytes, T cells are the main immune cells. It is accepted that thymoquinone, one of the bioactive components of black cumin oil, increases T cells and phagocytic cells mediating immune response and thus assumes an effective immune mediator role. It has also been reported that thymoquinone, which causes an increase in T lymphocytes, the strategic cells of the immune system, shows antibacterial effect against some bacteria including Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis. It has been shown that black cumin seed administered after infection of mice with Candida Albicans significantly suppressed the development of this fungus. Apart from bacterial and fungal infections, it has been reported that the bioactive content of black cumin seed has a strong antiviral effect against murine cytomegalovirus virus and eliminates the disease-causing virus (Salem and Hossain, 2000; Khan et all., 2003; Tjandrawinata et all., 2015; Güzelsoy, 2018).

## Antihistaminic Effect of Nigella Sativa

Histamine is a defense product and is a chemical released from mast cells and basophils. In all cases of exogenous and endogenous hypersensitivity and in common health problems such as atopic dermatitis, bronchial and atopic asthma and allergic rhinitis, suppression of the chemical substances that cause allergic reactions is a priority in medicine in terms of relieving symptoms and improving the quality of life until the agent is eliminated. In this context, the number of studies using black cumin seeds and its ingredients has increased. For example, black cumin oil administered to volunteer subjects for the treatment of bronchial asthma suppressed allergic symptoms in most patients. Black cumin oil is thought to be effective in this suppression. It is estimated that it may be effective in allergic reactions by decreasing immunoglobulin E and eosinophil count and plasma cortisol. Within the scope of the antihistamine effect of black cumin, it can also be said to have antiasthma effect. Bronchial asthma is a disease characterized by chronic airway inflammation and is a phenomenon in which leukotriene-mediated pathologies are observed. Studies suggest that the expression of 5-lipooxygenase, the main enzyme of leukotriene biosynthesis, is inhibited by thymoquinone. Thymoquinone has also been reported to suppress pulmonary inflammation by relatively decreasing defense cells in the respiratory tract (Taşar, 2010; Koshak et all., 2017; Yalçın, 2019).

## Epilepsy and Nigella Sativa

Convulsant factors and convulsive phenomena cause spasmic convulsive seizures. The most common of these phenomena is epilepsy. Anticonvulsants and antiepileptics are chemicals used in the prevention and treatment of epileptic seizures, bipolar disorder, and neuropathic pain. These chemicals prevent the neuron activity that causes convulsions and prevent the occurrence and recurrence of seizures. The anticonvulsant effect of black cumin and its bioactive components has been wondered and studies have been conducted in this context. In one study, the effects of valproate, an antiepileptic drug, and black cumin oil were compared clinically, and it was suggested that black cumin oil had a stronger anticonvulsant effect than valproate; it was reported that the anticonvulsant effect of black cumin oil could be utilized for treatment (Raza et all., 2008; Yalçın, 2019).

## DNA Damage and Black Cumin

In response to thousands of factors that adversely affect the genetic material and cause DNA damage, the organism has a powerful and efficient DNA repair system for DNA that is constantly attacked and damaged. Nevertheless, in the event of DNA damage due to unavoidable causes, cancers and other proliferative diseases can occur because of uncontrolled division or faulty cell division. Studies conducted to see how the bioactive components in black cumin affect cell DNA and cell structure indicate that black cumin seed extract can prevent DNA damage and has a strong protective effect especially against radioactivity-induced DNA damage (Yalçın, 2019).

## Anti-tumoral Effect of Nigella Sativa

Studies conducted in the process of searching for solutions to cancers, which is a global and increasingly widespread health problem, have also investigated the antitumoral effects of black cumin seed content. Many in vivo and in vitro studies have shown that the antitumoral effect is due to the thymoquinone in black cumin seed content. These studies suggest that thymoquinone significantly inhibits cell division in cancer cases such as breast and ovarian adenocarcinoma, colorectal cancers, neoplastic keratinocytes, osteosarcoma, fibrosarcoma, lung carcinoma, and prostate cancer, thus showing antitumoral effect. In mice treated with thymoquinone, it has been shown that the growth of tumor size was inhibited and even the size was reduced. In neutropenia cases in which neutrophil count decreases, neutropenia accompanied by high fever is febrile neutropenia, which is defined as a single oral measurement of body temperature >38.3 C or ≥38°C for more than 1 hour. Black cumin seeds were administered to children with brain tumors to investigate the preventive effects of black cumin on febrile neutropenia due to cancers, and it was noted that the incidence of febrile neutropenia decreased and the vital signs and quality of life of the patients increased. It has been accepted that thymoquinone can destroy cancer cells at G0/G1, G1/S, G2/M cell cycle points and that it is a phytochemical with anticancer properties (Asal et all., 2018; Güzelsoy, 2018; Yalçın, 2019).

## Antihypertensive Effect of Nigella Sativa

Among their wide spectrum of therapeutic effects, the antihypertensive effect of black cumin seeds and thymoquinone should also be considered. In a study investigating the blood pressure-regulating antihypertensive effect of thymoquinone, it was observed that arterial blood pressure decreased to the physiological range and heart rhythm returned to normal beats in hypertensive animals treated with black cumin extract. It is estimated that this effect of black cumin seeds is also supported by its diuretic (diuretic) property (Yalçın, 2019).

Black cumin, which attracts attention with its ability to generate impulses with a very complex network of effects, appears to be a structure that reveals unique therapeutic effects in each medical condition. This opinion is based on its therapeutic effects in many diseases. Apart from this, it is also used for flavor purposes, and its presence in foods creates a visual and sensory sympathy. As a spice, it is added to foods such as bread, buns, pastries, cakes, cheeses, pastas, salads, honey with its flavoring feature, and it is included in cosmetic products to protect hair health. The use of black cumin, which has a very long history, and the sympathetic perception of black cumin is an important infrastructure for the development of new products in a wide range from food production to the pharmaceutical industry. Its characteristics such as being an easy-to-grow, economical and natural plant, and its recommendation in the hadiths constitute strong scientific evidence, all of which increase the preference of the plant. Islamic societies have considered these recommended grains as an important stakeholder in life. These healing grains, which are also highly valued in our culture, are a black prescription for us. Many scientific studies, of which we have presented some examples, demonstrate the positive effects of black cumin seeds on human and animal health within the scope of evidence-based medicine. It is our hope that the studies with black cumin seed active ingredients will become targeted, and that the formulations obtained after completing the phase studies in the pharmaceutical process will take their place in pharmacies as original drug forms as soon as possible. The potential of black cumin seeds and oil for health and wellness is tangible. However, the scientific, intelligent, and conscious utilization of this plant and its active ingredients has not been sufficiently developed. It is a mistake to assume that plants and herbal products are safe under all circumstances. Even with black cumin seeds, it should be kept in mind that phyto therapeutic applications should be performed at the appropriate dose and time interval, and it is important to question plant-plant, plant-drug, or plant-disease interactions. Although black cumin seeds and oil are reported to have no toxic effects and very low toxicity, this sensitivity should be taken into consideration. Like all seeds and seeds, black cumin seeds are a rich source of vitamins and minerals, including strategic essential oils, amino acids, minerals such as magnesium, zinc, selenium and many antioxidants and vitamins. Plant antioxidants have recently gained attention as important dietary supplements with very low side effects. Black cumin seeds with their powerful antioxidant content should share in this popularity. The correct and practical way for those who want to use black cumin seeds is to buy fresh and high-quality black cumin seeds and use them by grinding them. Black cumin seed oil is also a product that can be used because it contains bioactive components. However, cold pressed black cumin seed oil from companies with national quality standard certificates such as TSEK, TÜRKAK or international quality standard certificates such as ISO 9000, ISO 14000 should be preferred. In fact, the most scientific solution is to turn the bioactive components of black cumin into medicines. For "thymoquinones" to be evaluated as active pharmaceutical ingredients, research is needed to better understand the effectiveness of thymoquinones. Nowadays, when the importance and vitality of domestic and patented medicine has been realized, it is expected that thymoquinone, the active ingredient of Nigella sativa L. seeds, will be developed as a new drug through clinical trials. It may be possible to update the classical formulations of black cumin used in traditional medicine for centuries and to produce new drugs that can be used in treatment. Another issue is the lack of sufficient studies on the use of black cumin essential oils as protective herbal antioxidants in foods. It is important to increase the use of essential oils in food technology. In recent years, more and more plant-based additives have been used to prevent physical, chemical, and microbiological deterioration in foods. Since herbal chemicals are natural, they carry a lower risk for human health. An important class of these additives are essential oils extracted from plants. In this context, black cumin essential oils can be investigated for each food and compared with synthetic antioxidants and the use of black cumin essential oils in foods can be expanded and a natural alternative to the use of synthetic antioxidants can be occurred (Gün, 2012).

**All-Time Medicinal Aromatic Plant: Nigella Sativa**

Black cumin seed, one of the phytotherapy elements of all times, has always been an important life companion for people in search of vitality, performance, health, and healing. The Prophet Muhammad's (PBUH) statement "There is no disease except death for which there is no cure in black cumin" and other hadiths in this sense emphasize that black cumin is a multifunctional source of healing. Due to the state of believing, which has the quality of advanced foresight and scientific acceptance, the Islamic society used black cumin with full confidence, believing in its benefits as well as its unrecognized cures. This approach also provided Islamic societies with the psychological serenity, peace and rehabilitation that comes from caring about and practicing something that the Prophet Muhammad (PBUH) cared about and recommended. In his book El-Kanun fi't Tıb, Ibn-i Sina described black cumin, which hundreds of Islamic physicians included in their preparations, as a plant that eliminates symptoms such as weakness and fatigue by activating metabolism (Gün, 2012;, Khan, 2003). In non-Islamic beliefs, the behavior of benefiting from the healing and even holiness of the black cumin plant draws attention. The fact that black cumin seeds were found in the tomb and mummy of Pharaoh Tutankhamon and that Cleopatra used black cumin oil for health and beauty shows that the plant is recognized and used not only in Islamic society but also in a wide range of areas. For example, ancient physicians such as Hippocrates and Dioscorides, known for their important work in the field of medicine and pharmacology, used black cumin oil and seeds in cases such as treatment of digestive system problems, toothaches and headaches, intestinal parasites, inflammation treatment, and sinus congestion (Gün, 2012; Yalçın, 2019). Scientific and pharmacological research in today's disciplines strives to make the ancient knowledge reported in hadiths as proven as possible. Thousands of studies have reported the antioxidant, antidiabetic, antibacterial, anticarcinogenic, antifungal, antitumoral, anticonvulsant, anti-inflammatory, antiulcerogenic, hypoglycemic and immune system strengthening effects of black cumin seeds and their bioactive components (Güzelsoy et all., 2018). Although it is an important medicinal aromatic plant, its effective use in drug formulations is not yet at the desired level. In addition, the awareness of its use in the hands of the public should be increased in line with provocative scientific studies.

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# CHAPTER II

# Sanggenon C Mıght Represent A Novel, Effectıve And Safe Antıoxıdant

**Zeyneb KARAKUŞ[1]**
**Halise Betül ASLAN[2]**

## Introduction

Antioxidants play a crucial role in mitigating the impact of free radicals that arise as a consequence of vital metabolic reactions (Akkuş 1995, Akpoyraz and Durak 1995, Delibaş and Özcankaya 1995, Young and Woodside 2001, Durmuş and Ünsaldı 2005, Akkoç 2008). They are part of the body's defense system designed to counteract the effects of these free radicals on macromolecules.

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The antioxidant defense systems serve several essential functions, including preventing reactive oxygen species formation, preventing damage caused by these species in metabolism, and facilitating detoxification processes. Antioxidants exhibit similar characteristics to free radicals and rapidly engage in reactions with them, effectively halting the progression of autooxidation and peroxidation reactions (Dündar and Aslan 2000, Yurttaş et al. 2000, Blokhina et al. 2003, Aslan 2018).

The effectiveness of the antioxidant defense system can be attributed to five distinct mechanisms: prevention of free radical formation, neutralization of generated free radicals, repair of cellular damage, cessation of secondary radical reactions, and augmentation of antioxidant capacity (Dündar and Aslan, 2000).

Antioxidants can be categorized into two primary groups: exogenous and endogenous antioxidants. Endogenous antioxidants can be subdivided into enzymatic and non-enzymatic categories (Oyman, 2014). Table 2.6 overviews the principal endogenous antioxidants and their respective roles in metabolism, while Table 4.7 delineates the primary exogenous antioxidants and their functions within metabolic processes (Aslan, 1999).

*Table1. Essential Endogenous Antioxidants and Their Functions*

| **Antioxidant** | **Location** | **Metabolic Function** |
|---|---|---|
| Cytochrome oxidase | Plasma | Neutralizes superoxide radicals |
| Superoxide dismutase | Mitochondria, Plasma, Peroxisomes | Converts superoxide to hydrogen peroxide |
| Catalase | Peroxisomes | Neutralizes H2O2, methyl, and ethyl hydroperoxides |
| Glutathione peroxidase | Cytosol, Mitochondria | Reduces lipid peroxidation products |
| Glutathione reductase | Cytosol, Mitochondria | Reduces low molecular weight disulfides |
| α-tocopherol (Vitamin E) | Membranes, Extracellular environment | Reduces peroxidation |

| | | |
|---|---|---|
| β-carotene (Vitamin A precursor) | Cell and tissue membranes | Acts as a peroxyl radical scavenger |
| Glutathione | Intracellular environment, alveoli | GSH redox substrate |
| Ascorbic acid (Vitamin C) | Intracellular and extracellular fluids | Regenerates Vitamin E |
| Cysteine | Widely distributed | Reduces organic compounds |
| Albumin | Plasma, Serum | Removes free radicals |
| Bilirubin | Blood, Tissues | Chain-breaking antioxidant |
| Ceruloplasmin | Blood, Tissues | Converts superoxide to H2O2 |
| Transferrin | Plasma | Binds iron ions |
| Lactoferrin | Plasma | Binds iron ions |
| Ferritin | Blood | Binds tissue iron ions |

*Table 2. Basic Exogenous Antioxidants and Their Functions*

| **Antioxidant Class** | **Specific Type** | **Function** |
|---|---|---|
| Xanthine Oxidase Inhibitors | Allopurinol | Inhibits the production of superoxide radicals in the xanthine oxidase reaction |
| | Oxypurinol | |
| | Tungsten | |
| Protease Inhibitors | Soybean Trypsin Inhibitor | Blocks the formation of xanthine oxidase from xanthine dehydrogenase |
| | Serine Protease Inhibitors | |
| | Phenylmethylsulfonyl | |
| NADPH Oxidase Inhibitors | Adenosine | Prevents superoxide formation by NADPH oxidase in neutrophils and macrophages |
| | Local Anesthetics | |
| | Calcium Channel Blockers | |
| | Nonsteroidal Anti-inflammatories | |

| | | |
|---|---|---|
| Superoxide Dismutase | Natural SOD | Catalyzes the dismutation of superoxide to H2O2 |
| | Polyethylene Glycol SOD | |
| | Ginkgo Biloba | Contains Fe-SOD with similar function and neutralization |
| Non-Enzymatic Radical Scavengers | Mannitol | Hydroxyl radical scavenger |
| | Dimethyl Sulfoxide | Collects Fe, superoxide, and hydroxyl radicals |
| | Spin Traps | Structures capable of scavenging all types of radicals |
| Iron Redox Chain Inhibitors | Deferoxamine | Prevents radical reactions by binding free Fe3+ atoms |
| | Apotransferrin | |
| | Ceruloplasmin | |
| Agents Enhancing Endogenous Defense | Antineutrophil Serum | Enhances cellular glutathione peroxidase enzyme activity |
| | Monoclonal Antibodies | Inhibits neutrophil adhesion to endothelium |
| | Platelet-Activating Factor | Inhibits neutrophil adhesion diffusion |

## A Novel and Important Antioxidant Potential: Phytochemicals

Phytochemicals are substances found in items such as vegetables, fruits, grains, seeds, and legumes. While they do not possess inherent nutritional value on their own, they contain various chemical properties that offer significant health benefits.

The everyday significance of phytochemicals has grown substantially, as numerous studies have substantiated their role in disease prevention. Experimental investigations into food constituents have led to the isolation and categorization of phytochemical compounds such as flavonoids, isoflavones, phytates, terpenes, polyphenols, lycopene, carotenoids, isothiocyanates, sulfites, and others. Some of these isolable phytochemicals are made available as concentrated preparations.

Healthcare professionals recommend and promote the consumption of many foods known to contain phytochemicals, as they have been shown to address issues like oxidative stress, cancer, cardiovascular problems, hypertension, diabetes, and more (Dündar 2001).

In this thesis, Sanggenon C, isolated from the root bark of Morus Alba L., is utilized. This flavonoid compound is known for its rich phytochemical content and various reported health benefits. Multiple studies have suggested that mulberry consumption may have positive effects on human health, particularly as a radical scavenger. Morus Alba contains a substantial amount of bioactive phenolic compounds (Sanchez-Salcedo et al., 2015).

**Versatile Antioxidants: Flavonoids**

In addition to well-established antioxidant vitamins, plants contain a diverse range of molecules with strong free radical scavenging properties, such as flavonoids, which exhibit equally potent antioxidant and hypolipidemic activities (Dündar 2001, Agati et al., 2012).

Flavonoids possess a 15-carbon atom 2-phenyl benzopyran (diphenylpropane) structure. Their general structural framework is depicted in Figure 2.6 (Pietta 2000). Plants are comprised of aromatic amino acids, phenylalanine, and tyrosine, along with malonate. The foundational structure of flavonoids consists of a flavan nucleus composed of 15 carbon atoms organized into three rings (C6-C3-C6). Depending on the variability in their skeletal structures, they are referred to by names such as flavone, flavonol, chalcone, and biflavonoid. Due to their structural attributes, they are categorized as polyphenolic compounds. Flavonoids are secondary metabolites in plants and originate from primary metabolites such as carbohydrates and amino acids.

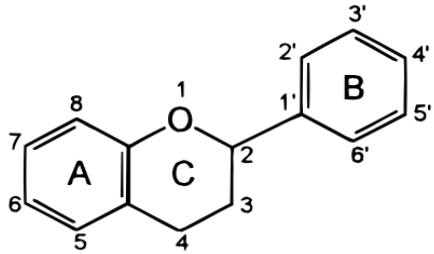


*Figure 1. The Flavonoid Structure*

Various flavonoids are formed by attaching hydroxyl (-OH) groups to the carbon atoms in the phenyl benzopyrone structure. The conjugation products are also formed by attaching groups such as sugar, methyl, sulfate, and similar groups to some of these -OH groups (Kahraman et al., 2002).

Flavonoids are typically found as glycosylated derivatives in plants, contributing to the vibrant shades of blue, red, and orange in leaves, flowers, and fruits. They are present in various vegetables, fruits, seeds, nuts, grains, spices, and different beverages (Pietta, 2000). Flavonoids exhibit diverse biological and pharmacological properties, including anti-cancer, anti-inflammatory, antimicrobial, antiviral, and immunomodulatory activities (Chen et al., 2017).

It has been observed that flavonoids inhibit enzymatic activities, catalyzing inflammatory reactions via phosphatidylinositol (PIP) in cells and preventing microsomal lipid peroxidation (Dündar, 2001). Flavonoids are reported to regulate cellular proliferation by suppressing phosphatidylinositol when necessary, and they positively affect the regulation of the cardiac conduction system with cardiac excitation (Weber et al., 1997).

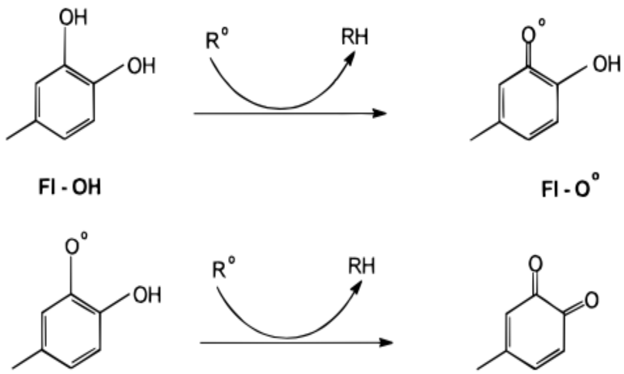


*Figure 2. Antioxidant Mechanism of Action of Flavonoids*

Flavonoids exhibit their antioxidant properties by engaging in reactions with free radicals. They inhibit cyclooxygenase and lipoxygenase enzymes and cleanse superoxide radicals ($O^{2-\cdot}$), hydroxyl radicals ($^{\cdot}OH$), and singlet oxygen ($_1O^2$). The antioxidant mechanism of action of flavonoids is depicted in Figure 2.7 (Pietta, 2000). They play a crucial role in cellular regulation through enzyme-dependent calcium modulation, inhibiting calmodulin. Flavonoids inhibit protein kinase activation and hinder lactate transport. Widely found in plants, flavonoids possess various biological and pharmacological properties, including anti-cancer, anti-inflammatory, antimicrobial, antiviral, and immunomodulatory activities (Chen et al., 2017).

They capture peroxyl radicals ($ROO^{\cdot}$) and alkoxyl radicals ($RO^{\cdot}$) and break the lipid peroxyl ($LOO^{\cdot}$) chain. The mechanism by which a flavonoid disrupts the lipid peroxidation chain reaction is likely as follows:

$PhOH + LOO^{\cdot} \rightarrow PhO^{\cdot} + LOH$

$PhO^{\cdot} + LOO^{\cdot} \rightarrow LOO\text{-}Ph(=O)$

In addition to their antioxidant effects, flavonoids have been identified through clinical studies to exhibit various metabolic effects. These effects can be categorized as antitumoral, antiviral, anti-inflammatory, antiallergic, vasodilatory, and immune system stimulating.

Flavonoids demonstrate their antitumoral effects by enhancing intercellular signaling, suppressing lactate transport, inhibiting calmodulin, regulating NO synthesis, and reducing cell proliferation. They exert antiviral effects by binding to viral proteins and blocking viral replication and cellular protein synthesis. Their anti-inflammatory effects are achieved by inhibiting mast cell and histamine release (Dündar, 2001; Rice-Evans, 2001; Serafini et al., 2010; Agati et al., 2012).

They manifest vasodilatory effects by regulating NO synthesis and antiallergic effects by inhibiting histamine release. Additionally, with their free radical scavenging properties, they prevent lipid peroxidation by inhibiting cyclooxygenase and lipoxygenase enzymes, thus preventing atherosclerosis and coronary heart diseases (Kahraman et al., 2002).

## A New Source of Flavonoids: Morus Alba L. (White Mulberry)

The Morus genus, comprising more than 150 species of mulberries, includes Morus alba L. (white mulberry) as one of its prominent examples. White mulberry is commonly used as a source of leaves to feed silkworms and ruminants. In some countries with temperate climates, including Turkey, it is cultivated for fruit consumption and traditional medicinal purposes. In Turkey, there are three prevalent species, ranked in terms of frequency as follows: White mulberry (M. alba), Black mulberry (M. nigra), and Red mulberry (M. rubra). It has been reported that white mulberry is naturally widespread throughout Turkey, followed by black mulberry and red mulberry (Lale & Özçağıran, 1996). The root bark of white mulberry is documented in the Chinese pharmacopeia (Sang-Bai-Pi), and the mechanisms of action of phenolic compounds isolated from its content provide a substantial source for various pre-clinical research (Nomura et al., 1983). Scientific research emphasizing the medicinal value of M. alba L. has focused on investigating its content. Rich in phenolic compounds, particularly flavonoids, and anthocyanins, it exhibits antimicrobial and free radical scavenging potential. Moreover, its role in disease prevention and treatment has been highly effective, with numerous publications highlighting its functions in atherosclerosis, diabetes mellitus, cancer, immune nutrition, and neuroprotective effects (Du et al., 2003; Butt et al., 2008; Venkatesh Kumar and Chauhan, 2008; Jung et al., 2015; Wu et al., 2020).

The Sanggenon family, isolated from the bark of Morus Alba L. and characterized as characteristic flavonoids, has gained significant attention in complementary medicine and pharmaceutical research in recent years. So far, approximately 60 Diels-Alder-type additions have been obtained from plants, particularly from the Morus family. The earliest discovered two Diels-Alder-type additions, Sanggenon C and D, are typical examples (Li et al., 2018). In addition to Sanggenon C and D, some other flavonoids isolated from the root bark of Morus Alba include Sanggenol Q, Sanggenol A, Sanggenol L, Kuwanon T, Cyclomorusin, Sanggenon F, Sanggenol O, Sanggenon N, three known Diels-Alder-type additions, Sanggenon G, Mulberrofuran G, and Mulberrofuran C, and a known benzofuran, Morasin E (Jung et al., 2015; Li et al., 2018).

The two stereoisomers of Sanggenon, Sanggenon C and D, can be considered antioxidants for protecting mesenchymal stem cells from oxidative stress. However, Sanggenon D has been reported to be more effective than Sanggenon C in electron transfer potential. Sanggenon C is more effective than Sanggenon D in radical scavenging, $Fe^{2+}$ binding, and cytoprotection potentials. These differences can be attributed to the stereoconfigurations of chiral atoms, particularly 3''-C (Li et al., 2018).

## Morus Alba's Bioactive Antioxidant: Sanggenon C

Sanggenon C ($C_{40}H_{36}O_{12}$) is a natural chemical member of the flavonoid class and is naturally found in the bark of the mulberry tree (Nomura et al., 1983; Venkatesh Kumar and Chauhan, 2008; Jung et al., 2015; Wei et al., 2016). Its IUPAC nomenclature is as follows: 2 - [(1S, 5S, 6R) -6- (2,4-dihydroxy benzoyl) -5- (2,4-dihydroxy phenyl) -3-methylcyclohex-2-en-1-yl] -1,3,8, 10a-tetrahydroxy-5a- (3-methylbut-2-enyl) - [1] benzofuro [3,2-b] chromen-11-one, as shown in Figure 2.8 (Wei et al., 2018).

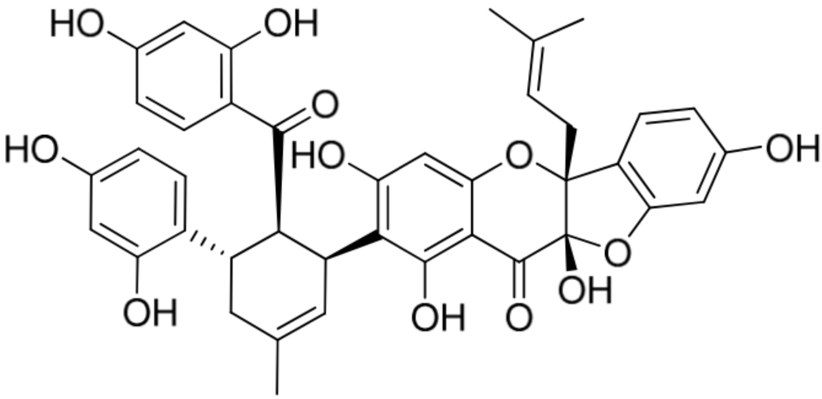


*Figure 3. Sanggenon C Structure*

## Sanggenon C: Physiological and Clinical Effects

The root bark of the mulberry tree (Morus alba L.) has been traditionally used in Chinese and Far Eastern medicine as an anti-inflammatory, liver-protective, kidney-protective, hypotensive, diuretic, cough suppressant, and analgesic agent (Nomura et al., 1983). It also exhibits antiviral and antimicrobial effects and has a neuroprotective role. Naturally occurring flavonoids, especially those in Morus Alba L. root bark extract, have shown antioxidant activity in various model systems (El-Beshbishy et al., 2006). Studies have demonstrated that Sanggenon C, one of the flavonoids isolated from white mulberry tree bark, has various bioactivities such as anti-cancer, anti-inflammatory, and cytoprotective effects against hypoxic damage in heart cells (Li et al., 2018).

In a study by Gu et al. (2017), Sanggenon C was reported to increase autophagy, protecting cardiomyocyte hypoxia. Huang et al. (2011) reported that Sanggenon C inhibits proteasome function, slowing cell cycle progression and inducing cell death in tumor cells. In a study by Dat et al. (2012), Sanggenon C was found to inhibit nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression, as well as suppress nuclear factor-κB (NF-κB) activity, offering protection against lipopolysaccharide (LPS)-induced inflammation in RAW264.7 cell culture. Li et al. (2002) reported that Sanggenon C inhibits tumor necrosis factor-alpha (TNF-α)-induced adhesion of polymorphonuclear leukocytes to human synovial cells and the expression of vascular cell adhesion molecule.

In a study by Chen et al. (2017), Sanggenon C was found to induce apoptosis in colon cancer cells by inhibiting nitric oxide production, nitric oxide synthase (iNOS) expression, and mitochondrial reactive oxygen species (ROS) activation. Wang et al. (2018) reported that Sanggenon C promotes osteoblastic proliferation and differentiation in a zebrafish model, prevents osteoclastic resorption, and improves prednisone-induced osteoporosis. Zhou et al. (2017) conducted a cell culture study and reported that Sanggenon C induces apoptosis in PC3 prostate cancer cells by activating caspase three and nine pathways.

In a study by Xiao et al. (2017) using a mouse model, they found that Sanggenon C protects against cardiac hypertrophy through the calcineurin/NFAT2 metabolic pathway.

Based on the data provided by the aforementioned scientific studies regarding the antioxidant, antitumoral, antiviral, and anti-inflammatory capacities of Sanggenon C, this research aims to evaluate its antioxidant and anti-inflammatory capacity in a different toxicity model.

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# CHAPTER III

# Digestive System Microbiata in Humans and Animals, Dysbiosis and Its Relationship with Metabolic Diseases

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**Tanay BİLAL**[2]

## Introduction

An animal organism is a superorganism consisting of roughly 10% body cells and 90% microbial cells located in this macroscopic host (Altuntaş & Batman, 2017). Intestinal microbiota is a community of microorganisms that function as a vital and very sensitive organ, consisting of billions of bacteria, viruses, fungi, protozoa and other protozoa that colonize the digestive system of humans and animals (Jarett & al., 2021). This microbiota is also

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called the second brain because it plays a key role in regulating the central nervous system (Yang & al., 2020). For this reason, maintaining and improving healthy microbiota activity is very important (Cakmak & İnkaya, 2021).

When we look at the types of microbiota in the body, there are the permanent microbiota, which is found throughout life and mostly continues its existence as a commensal, and the temporary microbiota, which stays in the body for hours or days, can cause the chemical or physical changes, and does not allow other microorganisms to shelter in the area (Cakmak & İnkaya, 2021). The surface area of bacteria settling in the human body is 400 $m^2$ (the size of a tennis court), and the bacterial mass is approximately 1.5 - 2 kg (Altuntaş & Batman, 2017). The microbiota has 10 times more cells and $10^{12}$ times more genes than the host's body (Kullük & Dalğın, 2021). The intestinal microbiota is like a fingerprint and each living thing has its own unique content and distribution (Altuntaş & Batman, 2017).

## Structure of Various Types of Microbiota

The human microbiota consists of primarily bacteria, viruses, fungi and many eukaryotic microorganisms (Cakmak & İnkaya, 2021). Bacteria in the flora contain a certain ratio of beneficial and harmful bacteria (Altuntaş & Batman, 2017). The average human gut contains approximately 100 million bacteria, and they make up a large portion of the human gut microbiota (Yuan & al., 2022).

There are 6 types of bacterial microbiota in the intestinal flora of healthy individuals, namely Firmicutes (gram-positive species such as *Clostridium*, *Eubacterium*, *Ruminococcus*, *Butyrivibrio*, *Anaerostipes*, *Roseburia*, *Faecalibacterium*), Bacteroidetes (gram-negative species such as *Bacteroides*, *Porphyromonas*, *Prevotella*), Proteobacteria (gram-negative family such as Enterobacteriaceae), Actinobacteria (gram-positive *Bifidobacterium* species), Fusobacteria and Verrucomicrobia (species such as *Akkermansia*) (Cakmak & İnkaya, 2021).

The presence of these bacterial groups in the intestine varies depending on various factors. The most numerically dominant bacterial groups are the anaerobic gram-positive Firmicutes and gram-negative Bacteroidetes groups (Özdemir & Büyüktuncer-Demirel, 2017). The sum of Bacteroidetes (9-42%) and Firmicutes (14-31%) constitute the majority of the microbiota (Kurtaran, 2021). Starting from infancy and towards old age, Firmicutes bacteria increase and Bacteroidetes decrease (Altuntaş & Batman, 2017).

*Lactobacillus* and Clostridia are generally dominant in the crop and gizzard parts of the digestive systems of poultry. *Lactobacillus*, *Streptococcus* and Coliforms are found in the duodenum. In the ileum, 68% of the microbiota is present and Lactobacillus, Clostridia, Streptococcus, butyrate-producing bacteria, Enterecoccus and Coliforms are generally dominant. Those in the cecum are *Lactobacillus*, Clostridia, *Bacteroides* and *Bifidobacterium*. It has been reported that bacteria that produce butyrate or are resistant to cellulose and starch digestion are commonly found in the cecum of good-performing broilers, whereas phylotype bacteria are common in the cecum of poorly performing broilers and most of them cannot be cultured (Tatlı-Seven & İflazoğlu-Mutlu, 2018).

In ruminants, most of the bacteria in the rumen are obligate anaerobes, but there are also facultative anaerobes and their number is between $10^7$ and $10^8$ (Tatlı-Seven & İflazoğlu-Mutlu, 2018). The majority of these bacteria consist of gram-negative bacteria (*Anaerovibrio lipolytica*, *Bacteroides ruminicola*, *Megasphaera elsdenii*, *Veillonella parvula*) (Özel & Sarıçiçek, 2009). Species such as *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are the most abundant cellulolytic bacterial species in the rumen. Rumen protozoa are divided into two groups: flagellates (Mastigophora) and ciliates (Ciliophora), but the majority are ciliates (Tatlı-Seven & İflazoğlu-Mutlu, 2018).

The dominant bacterial communities in the digestive tract of dogs and cats are Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and in cats also *Eubacterium* (Suchodolski, 2011). The gut microbiota of dogs is relatively similar to that of humans (Jarett & al., 2021). Clostridia are predominant in the duodenum and jejunum, and are found in high numbers in the ileum. Fusobacteria and *Bacteroides* live mostly in the ileum and colon. *Lactobacillu*s is commonly found in all parts of the small intestine of dogs at levels between $10^4$ and $10^8$ CFU/ml. *Lactobacillus acidophilus* is the dominant species among them (Grzeskowiak & al., 2015).

## Benefits of Microbiota

The microbiota has important functions in our body in regulating the development of diseases and health conditions, creating the necessary signals to promote the maintenance of immune system functions, and absorbing undigested carbohydrates. As a result of the research, it has been understood that the digestive system microbiota elements act as an organ with important functions in many metabolic events and systemic and mucosal immune system functions (Cakmak & İnkaya, 2021).

The microbiota in the gastrointestinal tract also has important roles such as removal of pathogenic microorganisms, production of antimicrobial factors, strengthening the mucosal barrier and immunoglobulin A induction (Stanley, Hughes & Moore, 2014). In addition, beneficial bacteria take part in biological and chemical processes such as the production of vitamins, short-chain free fatty acids and conjugated linoleic acid, synthesis of amino acids, biotransformation of bile acids, fermentation and hydrolysis of indigestible nutrients, modulation of the immune system, synthesis and detoxification of ammonia (Altuntaş & Batman, 2017).

The gastrointestinal tract microbiota enables the conversion of glycans into usable sugars through the production of glycoside hydrolase. In this way, they create short-chain fatty acids from the carbohydrates they digest (Kurtaran, 2021). Short-chain fatty acids are the major energy source of the colon epithelium and provide 10-15% of the energy of adults. In addition, the metabolic effects of short-chain fatty acids include increasing mucosal circulation, providing energy support to the mucosa, ensuring healing after resection procedures, increasing intestinal cell proliferation, stimulating water and sodium absorption, and increasing mucus production (Pedersen & al., 2016).

It has been proven that the presence of pets has a major role in the development of the microbiota of both adults and especially newborn children and reduces the incidence of allergic diseases such as asthma, allergic rhinitis and atopic dermatitis (Kullük & Dalğın, 2021). It is believed that healthy gut microbiota can aid human brain and neural development, as well as sustain brain function, mood, and cognition (Yang & al., 2020). Increasing bacterial diversity has positive metabolic effects such as decreasing hs-CRP, insulin resistance and leptin (Altuntaş & Batman, 2017).

Microbial communities in the intestines also play a very important role in maintaining the health of animals (Syromyatnikov & al., 2020). Microbiota plays a very important role in increasing the productivity of farm animals, maximizing feed utilization and increasing milk fat (Kullük & Dalğın, 2021).

## Prebiotics, Probiotics and Postbiotics

Healthy nutrition recommendations including prebiotic and probiotic foods and probiotic agents are promising in the treatment of metabolic syndrome and cardiovascular diseases (Altuntaş & Batman, 2017). In poultry, probiotics and prebiotics are very important in that they stimulate changes in the composition of the digestive tract microbiota population and provide great benefits to host health (Tatlı-Seven & İflazoğlu-Mutlu, 2018). It is known that intestinal microbiota has the potential to positively affect the health status of cats and dogs, depending on the use of probiotics and/or prebiotics (Ural & al., 2019).

Prebiotics are nondigestible nutrients or chemicals that have the ability to selectively stimulate one or more types of bacteria in the intestinal microbiota of humans and animals (Kurtaran, 2021). They cause the pathogenic bacteria in the intestine to decrease and their numbers to increase by creating a substrate for beneficial microorganisms. Galactooligosaccharides, fructooligosaccharides and mannanoligosaccharides are prebiotics (Tatlı-Seven & İflazoğlu-Mutlu, 2018). Foods such as bran sources, raw asparagus, bananas, endive, onions, garlic and leeks contain these molecules that enable the proliferation of *Bifidobacterium* and *Lactobacillus* strains, which have positive metabolic effects in the gastrointestinal environment (Kurtaran, 2021).

It has been observed that prebiotics have a positive contribution to metabolic markers by changing the microbiota composition (Altuntaş & Batman, 2017). Short-chain fatty acids, which are formed as a result of the fermentation of indigestible carbohydrate components, which also have prebiotic properties, by bacteria in the microbiota, are both an energy source for colonocytes and can improve health by showing anti-inflammatory, anticarcinogenic and immunomodulatory effects (Özdemir & Büyüktuncer-Demirel, 2017). It has been shown that in infants consuming formula prepared with galactooligosaccharide/fructooligosaccharide mixture, Bifidobacteria and secretory immunoglobulin A increased, pathogenic microorganisms, fecal pH and atopic diseases decreased, infection rates decreased and defecation patterns improved (Kurtaran, 2021). In a study (Roopchand & al., 2015), dietary polyphenols (grapes and cranberries) increased *Akkermansia muciniphila* bacteria in the mouse intestine and reduced all parameters related to metabolic syndrome by reducing the Firmicutes/Bacteroidetes ratio.

Probiotics are live microbial supplements used by animal producers to protect animals from enteric pathogen infections and improve animal health (Tatlı-Seven & İflazoğlu-Mutlu, 2018). *Lactobacillus* species, *Bifidobacterium* species, *Streptococcus salivarius*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Escherichia coli*, *Clostridium butyricum* and *Saccaromyces boulardii* are among the probiotics used for this purpose (Kurtaran, 2021). Probiotics provide improvements in metabolic parameters as a result of direct enzymatic and metabolic effects such as antitoxigenic, antimicrobial and anti-inflammatory effects, regulation of intestinal microbiota, and modulation of the immune system (Altuntaş & Batman, 2017). They prevent the acidification of the colon by competing with pathogenic microorganisms (Kurtaran, 2021). Another important effect of probiotics is to prevent pathogenic microorganisms from adhering to the intestinal wall (Tatlı-Seven & İflazoğlu- Mutlu, 2018). Consumption of cheese and milk reduces the level of choline and trimethylamine-N-oxide in urine and increases the excretion of acetate, propionate and lipids in feces (Altuntaş & Batman, 2017).

It has been determined that probiotic foods such as cheese, yoghurt, fermented milk and soy milk have angiotensin converting enzyme inhibition activity (Altuntaş & Batman, 2017). Many studies have shown that the use of probiotics improves intestinal microbiota fermentation, reduces hunger, improves postprandial glucose response, and causes an increase in glucose tolerance along with weight loss and a decrease in calorie intake. Apart from obesity, probiotic applications have gained importance for urogenital system diseases, *Helicobacter* gastritis and parasitic infections, as well as in the treatment of inflammatory bowel infections (Grzeskowiak & al., 2015). In a meta-analysis of 12 randomized controlled trials investigating the effects of probiotics on type 2 diabetes patients, fasting blood sugar, HDL cholesterol, HOMA-IR, HbA1C levels improved significantly as a result of treatment (Li & al., 2016). Fasting blood sugar and HbA1C decreased significantly in type 2 diabetics who consumed 300 g/day of probiotic yoghurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* for six weeks (Yadav, Jain & Sinha, 2007). *Lactobacillus rhamnosus* as a probiotic caused weight loss and a decrease in white adipose tissue when administered to obese mice for eight weeks (Lee & al., 2006). There was a significant decrease in total cholesterol and LDL cholesterol levels in 485 patients as a result of a probiotic-rich diet (Guo & al., 2011). Daily consumption of certain probiotic strains significantly reduced vulnerability to depression, anxiety and bad mood (Yang & al., 2020).

Symbiotics are the application of prebiotics and probiotics together. Postbiotics are a relatively new term and are metabolic products (e.g. hydrogen peroxide) of probiotic bacteria (especially *Bifidobacterium breve* C50 and *Streptococcus thermophilus*). There are studies showing that postbiotics increase the immunoglobulin A response and thymus size to the polio vaccine in infants, prevent gastroenteritis and allergy, and reduce inflammation (Kurtaran, 2021).

## Formation of Microbiota at an Early Age

The composition and function of the intestinal microbiota, which consists of many microorganisms such as bacteria, viruses, fungi and protozoa, are affected by various factors such as mode of birth, breast milk intake, antibiotic use and nutrition. The first dietary factor affecting the microbiota is breastfeeding because it is well known that oligosaccharides, lysosome, lactoferrin, antibodies and cytokines found in breast milk increase the number of *Bifidobacterium* in the intestine (Özdemir & Büyüktuncer-Demirel, 2017). After one year of age, the intestinal microbiota becomes similar to the digestive system microbiota of a young person. It is accepted that the intestinal microbiota reaches its adult microbiota composition at an average age of 2-3 years (Cakmak & İnkaya, 2021).

Breast milk is a symbiotic food that contains prebiotics (breast milk oligosaccharides) and probiotics (*Bifidobacterium*, *Lactobacillus*) (Özdemir & Büyüktuncer-Demirel, 2017). It has been found that *Lactococcus* levels in the intestines of exclusively breastfed babies are lower than formula-fed babies. It has been shown that babies receiving both breast milk and formula have similar microbiota composition to babies fed only formula (Madan & al., 2016). Exposure to microbes at an early age affects the infant's gut microbiota and the child's vulnerability to various diseases. Antibiotic exposure, cesarean birth, and formula feeding may prevent the establishment of an appropriate infant microbiome (Yang & al., 2020).

## Factors Affecting the Microbiota

Microbiota varies depending on endogenous and exogenous factors that change throughout a person's life, such as geographical origin, genetics, type of birth, age, lifestyle, nutrition, antibiotic use and previous diseases. Changes in microbiota distribution also occur due to diet, host genetics and viral infections (Altuntaş & Batman, 2017). It has been determined that antimicrobial and methane-reducing feed additives are effective on rumen fermentation and microbiota (Tatlı-Seven & İflazoğlu-Mutlu, 2018). Factors affecting the human microbiota are maternal colonization, age, diet, environmental contacts and antimicrobial treatments (Kurtaran, 2021). Animals exposed to unpredictable chronic mild stress conditions such as restraint, nocturnal lighting, isolation, food or water deprivation, dirty cage, and cage tilting for several weeks exhibited dysregulated gut microbiota and metabolic findings (Rincel & al., 2019).

The toxicological significance of the interaction of the intestinal microbiota with pollutants is a serious concern because chemicals that interrupt the functions of the intestinal microflora lead to changes in the homeostasis of animals. High doses of fungicides disrupt the metabolism of mice by altering the intestinal microbiota (Syromyatnikov & al., 2020). Antibiotic use directly affects the intestinal microbiota, reducing the number and diversity of bacteria. In turn, the gut microbiota altered by antibiotics may affect the chemical transformation of xenobiotics in the body (Koppel, Rekdal & Balskus, 2017). Additionally, various classes of pesticides (insecticides, fungicides, and herbicides) can affect the intestinal microbiota of animals. Changes in the microbiome caused by pesticides ultimately affect the animals' immunity, reproductive ability, and even behavioral traits. Glyphosate has a significant negative effect on the intestinal microbiota of both mammals and plant pollinators (Syromyatnikov & al., 2020).

*Akkermansia muciniphila* is a gram-negative mucin-degrading bacterium and constitutes 3-5% of the intestinal microbiota. Intestinal *Akkermansia muciniphila* levels are inversely associated with obesity and type 2 diabetes (Altuntaş & Batman, 2017). It was observed that the density of the anti-obesity bacteria *Akkermansia muciniphila* was higher in athletes who exercise regularly compared to the control group (Clarke & al., 2014). *Akkermansia muciniphila* concentration increases when taking antidiabetic drugs such as metformin. Administration of *Akkermansia muciniphila* to mice increases weight loss and improves metabolic parameters such as hyperglycemia and adipose tissue inflammation (Altuntaş & Batman, 2017).

## Effect of Diet

The diet is the substrate for microbial growth, enabling the production of microbial metabolites such as short-chain fatty acids, amino acids, vitamins and secondary bile acids. Diet has a strong impact on the gut microbial community and can therefore influence various aspects of animal health (Jarett & al., 2021). It is seen that the main dietary factors affecting the microbiota in adulthood are the carbohydrate (especially fiber), protein and fat content of the diet (Özdemir & Büyüktuncer-Demirel, 2017). The structure and amount of proteins, fats and carbohydrates in the diet greatly affect the composition of the host's digestive microbiota (Tatlı-Seven & İflazoğlu-Mutlu, 2018).

It is stated that the Mediterranean diet improves obesity, inflammation and lipid indicators, the vegetarian diet increases microbiota diversity, and the gluten-free diet has effects that increase the number of beneficial bacteria and reduce the number of harmful bacteria (Melini & Melini, 2019). It has been determined that high-fiber and plant-based diets increase the bacterial diversity of the intestine and *Prevotella* and *Xylanibacter* species (Özdemir & Büyüktuncer-Demirel, 2017). Proteins, one of the main elements of the diet, are an important food source for microorganisms. For example, *Lactobacillus* cannot synthesize the amino acids necessary for their anabolism, and therefore the increase in the number of these bacteria in the environment largely depends on amino acids (Tatlı-Seven & İflazoğlu- Mutlu, 2018). Proteins reaching the colon are not absorbed by colonocytes, unlike the enterocytes in the small intestine, but are fermented by the bacterial microbiota and transformed into various metabolic products (Altuntaş & Batman, 2017). Some of the bioactive metabolites formed as a result of bacterial fermentation are hydrogen sulfate, ammonia, aromatic compounds (phenol, p-cresol, indole), polyamines (agmatine, putrescine, spermidine, spermine, cadaverine), short-chain fatty acids, organic acids (lactate, formate, succinate), ethanol, gases ($H_2$, $CO_2$, $CH_4$) and compounds with potential neuroactive effects (GABA, serotonin, histamine, L-DOPA, triamine, nitric oxide, tryptamine, phenethylamine) (Davila & al., 2013). It is suggested that these metabolites formed as a result of a high-protein diet cause colorectal cancer, inflammatory bowel disease and atherosclerosis (Windey, De Preter & Verbeke, 2012).

It has been shown that diets high in animal protein and saturated fat and low in fiber and carbohydrates reduce the richness and diversity of the intestinal microbiota and increase Firmicutes and Proteobacteria colonization (Özdemir & Büyüktuncer-Demirel, 2017). The most important energy source of the microbiota is carbohydrates taken with the diet. Volatile fatty acids such as acetate, propionate and butyrate are formed from ingested and indigestible polysaccharides through fermentation (Altuntaş & Batman, 2017). It has been observed that a diet rich in saturated fatty acids contributes to the development of hepatic steatosis and obesity and increases the Firmicutes/Bacteroidetes ratio in the intestinal microbiota (De Wit & al., 2012). Conjugated linoleic acid is produced in the rumen of ruminants as a result of the biohydrogenation of unsaturated fatty acids in the feed. Conjugated linoleic acid tends to increase serum insulin and glucose in non-diabetic animals and humans, while decreasing serum insulin, glucose and fatty acid levels in diabetic rats. Conjugated linoleic acid has anti-carcinogenic, anti-obesity, anti-diabetic and antiatherogenic effects (Churruca, Fernandez-Quintela & Portillo, 2009). It has been reported that animal fats increase the number of *Clostridium perfringens* in the ileum of broilers more than soybean oil (Tatlı-Seven & İflazoğlu-Mutlu, 2018).

The pathogenicity of some gram-negative bacteria with pathogenic properties is due to the content of endotoxin-like lipopolysaccharide in the cell wall (Cakmak & İnkaya, 2021). As a result of high-fat nutrition, mucosal integrity is disrupted and wall permeability and plasma lipopolysaccharide levels in intestinal cells increase (Altuntaş & Batman, 2017). The increase in plasma lipopolysaccharide level is effective in increasing basal inflammation and creating environments that are conducive to metabolic diseases (Rodes & al., 2013). The presence of some lipopolysaccharide in the blood of healthy people indicates that lipopolysaccharides are constantly absorbed from the intestine in low amounts, and in cases with obesity and type 2 diabetes, increased lipopolysaccharide levels in the plasma as a result of high-fat nutrition indicate the presence of inflammation (Cakmak & İnkaya, 2021). The microbiota suppresses the fasting-stimulated adipose tissue factor, causing an increase in lipopolysaccharide activity and suppression of peroxisomal proliferator-activated receptor co-activator-1, which is involved in beta-oxidation (Altuntaş & Batman, 2017). It has been shown that at the end of this process, the formation of metabolic diseases such as obesity and diabetes accelerates (Bäckhed & al., 2004).

**Dysbiosis**

The microbiota composition of the digestive system can be disrupted due to factors such as the lifestyle choices such as diet, genetic structure and smoking, the exposure to potentially pathogenic microorganisms, the antibiotic use and the stress. Disturbance of the balance of the microbiota is called dysbiosis (Kullük & Dalğın, 2021). It has been reported that the microbial dysbiosis process, in which the beneficial/harmful bacteria ratio is disrupted, is associated with many diseases such as allergy, inflammatory bowel disease, lupus, asthma, multiple sclerosis, Alzheimer's, Parkinson's disease, celiac disease, hypertension, cardiovascular diseases and atherosclerosis (Yılmaz & Altındiş, 2017). There are also studies showing a relationship between dysbiosis and metabolic syndrome, obesity, type 2 diabetes, non-alcoholic fatty liver disease and some types of cancer (Tilg & al., 2018). Multiple gastrointestinal disorders, such as respiratory allergies, atopic dermatitis, and chronic enteropathy and inflammatory bowel disease, can be triggered due to dysbiosis (Tatlı-Seven & İflazoğlu-Mutlu, 2018). In microbial dysbiosis, harmful metabolites increase through the fermentation of carbohydrates and proteins and changes occur in the composition of bile acids (Altuntaş & Batman, 2017).

It has been reported that changes in the intestinal microbiota may cause the modulation of signaling pathways and the release of proinflammatory cytokines, which will indirectly shape the secretion of amyloid and lipopolysaccharide (Jiang & al., 2017). Dysbiosis may play a role in the basis of many different neurological disorders as well as neurodegeneration (Ural & al., 2019). It has been reported that the microbiota affects the immune and digestive systems in dogs and cats, and disruptions in microbiotic balance can cause inflammatory and allergic diseases (Tatlı-Seven & İflazoğlu-Mutlu, 2018). These disorders are common in both cats and dogs, and a prevalence of up to 17.8% has been reported (Jarett & al., 2021). It has been shown that there is a positive correlation between the presence of *Ralstonia picketti* in visceral tissue and systemic inflammation, and between its fecal level and insulin resistance (Kurtaran, 2021). Another situation where the beneficial/harmful bacteria ratio is disrupted is the use of antibiotics (Cakmak & İnkaya, 2021). Antibiotic use causes temporary or permanent microbial dysbiosis, depending on the type and age at which it is used (Altuntaş & Batman, 2017).

## The Relationship Between Dysbiosis and Obesity

Unbroken polysaccharides, which occur in individuals fed with high protein and carbohydrates, undergo fermentation by the microbiota (Cakmak & İnkaya, 2021). *Bacteroides thetaiotaomicron* is one of the dominant members of the flora and provides 10-15% of the calorie requirement by digesting polysaccharides that cannot be digested in any other way (Kurtaran, 2021). It is reported that short-chain fatty acids formed as a result of fermentation are 20% more in obese than in lean people (Cakmak & İnkaya, 2021). Short-chain free fatty acids are direct energy providers of the colon epithelium and stimulators of adipogenesis through their absorption into the portal circulation. *Lactobacillus* species reduce fat absorption by preventing the binding of bile acids (Kurtaran, 2021).

As a result of the deterioration in mucosal integrity that occurs as a result of high-fat nutrition, wall permeability and plasma lipopolysaccharide levels in intestinal cells increase. Since the intestinal permeability of obese people is higher than that of non-obese people, it causes a constant inflammatory stimulus. Obesity-induced inflammation is chronic and low-level and occurs as a result of excessive intake of nutrients in metabolic cells. The subsequent inflammatory response is managed by metabolic cells, leading to the activation of specialized immune cells and an inflammation that does not resolve in the tissue (Cakmak & İnkaya, 2021). In obese patients, lipopolysaccharides formed as a result of fatty diet increase proinflammatory macrophages and proinflammatory cytokines such as TNF-α and IL-6 in adipose tissue (Xu & al., 2003). In obesity, adipose tissue increases and in this process, the release of mediators that have directive functions in the endocrine and immune systems, such as pro-inflammatory cytokines and the leptin hormone, increases. This inflammation process damages the working mechanism of insulin, insulin sensitivity decreases, and food disrupts energy metabolism (Cakmak & İnkaya, 2021).

It has been observed that *Lactobacillus plantarum* and *Lactobacillus curvatus* treatment reduces fat accumulation and proinflammatory cytokines in the adipose tissue in obese people (Yoo & al., 2013). It was determined that the density of the anti-obesity bacteria *Akkermansia muciniphila* was higher in athletes who exercise regularly compared to the control group (Clarke & al., 2014). In the microbiota of obese people, it is generally observed that the rate of Firmicutes increases and the rate of Bacteroidetes decreases, similar to aging and colorectal cancer (Ley & al., 2005). It has been determined that obese mice contain 50% less Bacteroidetes and 50% more Firmicutes species in their intestines than lean mice. The world antibiotic use map and the obesity map also show similarities (Kurtaran, 2021).

## The Relationship Between Dysbiosis and Diabetes

It is reported that factors such as diet, physical inactivity, and unbalanced nutrition, as well as changes in the intestinal microbiota, are effective in the development of insulin resistance (Kamarlı-Altun & Akal-Yıldız, 2018). As a result of changes in intestinal permeability due to disrupted intestinal microbiota, lipopolysaccharides are absorbed by enterocytes during chylomicron secretion, enter the systemic circulation and cause subclinical inflammation. It is reported that eating foods containing high amounts of fat increases the ratio of gram-negative/gram-positive bacteria, and this is effective in the development of insulin resistance, by increasing the amount of lipopolysaccharide and therefore endotoxemia and subclinical inflammation (Yetkin, Yetiş & Kayahan-Satış, 2018). It has been shown that increased plasma lipopolysaccharide and insulin levels are associated with diabetes, as in obesity, and even lipopolysaccharide levels are much higher in type 2 diabetes (Creely & al., 2007). As a result of suppression of insulin secretion, pancreatic-duodenum homebox-1 mRNA production decreases and insulin resistance occurs (Altuntaş & Batman, 2017). In addition, as a result of all these events, autoreactive T cell regulation is disrupted, and this may cause beta cell damage in the pancreas and type 1 diabetes through autoimmune reactions (Vaarala, 2013).

## The Relationship Between Dysbiosis and Immunity

The balance of the intestinal microbiota is achieved by keeping the microbial composition that provides immune regulation in balance, that is, by symbiosis. The human microbiome is in close interaction with the immune system on all epithelial surfaces (Kurtaran, 2021). It has been determined that autoimmune events are less common in countries where helminth infestations are intense (Emoto & al., 2017).

In genetically predisposed individuals, some commensal bacteria play a potential trigger and maintenance role in inflammatory arthritis. Isolation of oral microbiota elements in synovial fluid has been demonstrated in rheumatoid arthritis and some spondyloarthropathy patients. The relationship between *Porphyromonas gingivalis* and rheumatoid arthritis is strongly shown in the literature. This factor produces peptidyl arginine deaminase, which citrullinates endogenous peptides (such as fibrinogen and alpha enolase peptides), and increased antibodies (anti-citrulline protein antibody) against citrullinated proteins have been shown in rheumatoid arthritis patients. It has been reported that periodontitis and tooth loss develop more in rheumatoid arthritis patients than healthy controls, that the severity of periodontal disease is related to rheumatoid arthritis disease activity, and that periodontitis treatment reduces rheumatoid arthritis activity (Kurtaran, 2021). A decrease in the amount of commensal bacteria (such as *Bifidobacterium* and *Bacteroides fragilis*) has also been shown in patients with rheumatoid arthritis (Maeda & Takeda, 2019). It is theorized that bacteria such as *Klebsiella pneumonia*, *Bacteroides vulgatus*, and sulfate-producing *Desulfovibrio desulfuricans* may be responsible for the pathogenesis of spondyloarthropathy and ulcerative colitis (Gopalakrishnan & al., 2018).

## The Relationship Between Dysbiosis and Atherosclerosis

Gut microbiota plays a key role in cardiovascular diseases (Tatlı-Seven & İflazoğlu- Mutlu, 2018). It can be thought that there are endotoxemia and basal inflammation resulting from microbiota dysbiosis in the common etiopathogenesis of pathophysiologically intertwined cardiometabolic diseases (Altuntaş & Batman, 2017). When the stools of patients with unstable atheroma plaques (patients who had a stroke) were examined, it was observed that the microbiome ecology changed, *Roseburia* species decreased, pro-inflammatory peptidoglycan-producing microbiomes increased, and anti-inflammatory carotene production decreased (Karlsson & al., 2012).

Poor oral hygiene has been associated with the risk of cardiovascular disease, and it has been suggested that oral flora may be the source of bacteria in atherosclerotic plaques (Cakmak & İnkaya, 2021). The observation that many of the bacterial products in atherosclerotic plaques are the same as human oral and intestinal bacteria has suggested that bacteria in this region may cause cardiovascular diseases as a source of atherosclerotic plaques (Koren & al., 2011). It has been shown that the oral pathogen *Porphymonas gingivalis* is frequently found in atheroma plaques and that the development of atherosclerosis is accelerated as a result of direct application of *Porphymonas gingivalis* to mice (Kozarov & al., 2005).

One of the mechanisms by which a protein-rich diet accelerates atherosclerosis is the increase in trimethylamine-N-oxide levels (Altuntaş & Batman, 2017). When L-carnitine, choline and phosphatidycholine are converted to trimethylamine by the microbiota in the intestines, trimethylamine freely enters the bloodstream and is oxidized by hepatic flavin monooxygenases to form trimethylamine-N-oxide. Excessive elevation in trimethylamine-N-oxide levels and the formation of uremic toxins lead to dysbiosis, resulting in the development of arteriosclerotic plaques and cardiovascular diseases (Tatlı-Seven & İflazoğlu-Mutlu, 2018). The increase in plasma trimethylamine-N-oxide varies depending on the microbiota composition, and it has been shown that the risk of atheroclerosis increases with its increase. Trimethylamine-N-oxide interacts with platelets, causing hyperactivity, which creates a tendency to thrombus (Cakmak & İnkaya, 2021). Another proatherosclerotic mechanism is that trimethylamine-N-oxide suppresses reverse cholesterol transport (Altuntaş & Batman, 2017).

**The Relationship Between Dysbiosis and Hypertension**

Increased blood pressure is possible as a result of the hormonal effects of short-chain fatty acids of the microbiota, increased basal inflammation with lipopolysaccharide, disrupting endothelial functions, and trimethylamine-N-oxide causing atherosclerosis (Altuntaş & Batman, 2017). It has also been observed that stimulation of short-chain fatty acid receptors in the smooth muscle cells of the kidney and blood vessels can affect blood pressure through the renin-aldosterone system (Miyamoto & al., 2016).

Tyrosine is converted to tyramine by the aromatic-L-amino acid decarboxylase enzyme produced by intestinal bacteria. It is thought that excessive production of tyramine, which occurs as a result of microbiota dysbiosis, may be responsible for the etiopathogenesis of essential hypertension. *Lactobacillus bulgaricus* produces histamine, tyramine and tryptamine, *Enterococcus faecalis* produces tyramine and *Lactobacillus plantarum* produces histamine and tyramine. An increase in the proportion of Firmicutes and Bacteroidetes has been reported in angiotensin II-induced hypertensive rats (Kurtaran, 2021).

**Dysbiosis, Nervous System and Depression**

Considering the interrelationship of the microbiota with the host, the microbiota gut-brain axis terminology should be used (Ural & al., 2019). The brain and intestine are involved in various pathways, including the enteric nervous system, vagus nerve, immune system or metabolic functions of host microorganisms (Zhu & al., 2017). There are three different basic mechanisms that govern the communication between the intestine and the brain: a) direct neuronal communication, b) hormonal-based signal communication tools and c) immune system (Westfall & al., 2017). From this point on, leaky gut stimulated by dysbiosis and therefore increased permeability directs or affects neurodegenerative disorders (Ural & al., 2019).

Wong & al. (2016) found that gut microbiota influences brain function and thus depressive and anxiety-like behaviors through inflammation-modulated signaling pathways. Some specific types of the gut microbiome can lead to depressive disorders by reducing the amount of neurotransmitters in the brain, and depression can also worsen symptoms by affecting the gut microbiome (Yang & al., 2020). Jiang & al. (2015) reported altered fecal microbial composition in patients with major depressive disorder, characterized by higher numbers of Enterobacteriaceae and *Alistipes* but fewer numbers of *Faecalibacterium*.

It is widely accepted that microbiota-derived short-chain fatty acids, represented by acetate, propionate, and butyrate, are vital mediators in the gut-brain axis. These metabolites may function as antidepressant therapeutics or adjuvants (Van de Wouw & al., 2018). Antibiotics and probiotics can significantly alter depressive-like behavior in both mice and rats, suggesting that the antidepressant effect of probiotics is mediated by the gut microbiota (Rieder & al., 2017). Probiotics appear to have the ability to improve the therapeutic effects of established chemotherapeutic agents for depression, particularly treatment-resistant depression (Yang & al., 2020).

## The Relationship Between Dysbiosis and Cancer

High amounts of fat and red meat predispose to cancer by increasing the levels of N-nitroso compounds and heterocyclic aromatic amines (Kurtaran, 2021). It has been stated that the microbiota contributes to carcinogenesis in distant organs through systemic inflammation, oxidative stress and epithelial genotoxicity regulated by tumor necrotizing factor (Salman & al., 2015). It has been reported that *Fusobacterium nucleatum* in patients with colorectal cancer, *Helicobacter pylori* in patients with stomach cancer, and *Porphyromonas gingivalis* and *Capnocytophaga gingivalis* located in the oral mucosa are effective in the development of cancer (Karpinski, 2019). It is frequently mentioned that *Porphyromonas gingivalis* and *Fusobacterium nucleatum* have an effect on the formation of colorectal and pancreatic cancer by causing the development of precancerous structures in the epithelial area (Fan & al., 2018). It is reported that *Candida albicans* colonization in the colon, which develops as a result of antibiotic use, paves the way for the formation of various cancers (Salman & al., 2015). Today, the most well-known microorganism that has an effect on the development of cancer is *Helicobacter pylori* (Karpinski, 2019).

Studies using mouse models have found that gut bacteria are responsible for the effectiveness of immunotherapy in the treatment of various types of cancer, including colorectal cancer (Yuan & al., 2022). In animal studies, *Bacteroides* and *Clostridium* species accelerate colon tumor growth, and *Lactobacillus* and *Bifidobacterium* species reduce tumor growth. The risk of cancer is high in the presence of *Bacteroides vulcatus* and *Bacteroides stercoris* and low in people with high *Lactobacillus acidophilus* (Kurtaran, 2021).

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# Current Overview of Herbal Antioxidants and Research

Black cumin seed has been used for thousands of years for the treatment of diseases, flavor and aroma. Today, it is widely used in pharmacy, food industry and cosmetics due to its antioxidant, antiviral and antibacterial effects. The biological active components, physiological and clinical effects of black cumin seed, especially antioxidant, anti-inflammatory, antidiabetic, antihistamine, anticonvulsant, anti-tumoural and antihypertensive mechanisms of action are discussed in detail.

Antioxidants play a role in detoxification of free radicals that develop during metabolic reactions and prevent harmful effects on the organism. Plant-derived antioxidant compounds have long been investigated for their beneficial effects in disease prevention. The effects of sanggenon C as a new, effective and safe antioxidant as a flavonoid compound obtained from the root bark of white mulberry have attracted attention in complementary medicine and pharmaceutical research in recent years. In this context, the antioxidant, antitumor, antiviral and anti-inflammatory effects of sanggenon C have been extensively investigated.

Basic behavioral, biochemical and physiological information about laboratory animals, especially rats, mice, rabbits, guinea pigs, hamsters have an important role in biomedical researches of all researchers. Knowing the anatomy, physiology, hematological and biochemical parameters of laboratory animals will enable researchers to know the animal they are working on, to ensure animal welfare, to understand the findings obtained, to care and feed animals under appropriate conditions, to prevent diseases and thus to carry out studies correctly and successfully.

This book is presented to its readers as a basic resource containing up-to-date information that all researchers working in the field of health can benefit from.

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