T.C.
REPUBLIC OF TURKEY
MARMARA UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

IN-VIVO INVESTIGATION OF THE PROTECTIVE EFFECTS OF TURKISH TRADITIONAL MEDICINAL PLANTS ON ACUTE INFLAMMATION

ABDUL HAMID
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PROF. DR. HATİCE KÜBRA ELÇİOĞLU
PHARMACOLOGY MASTER PROGRAM

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Tez Başlığı : Türk Geleneksel Tıbbi Bitkilerin Akut İnflamasyon Üzerindeki Koruyucu Etkilerinin İn-vivo Araştırılması

Bu çalışma, içerik ve kalite bakımından Yüksek Lisans Tezi olarak kabul edilmiştir.

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Bu tez, yukarıda isimleri bulunan jüri üyeleri tarafından ”Marmara Üniversitesi Lisansüstü Eğitim Öğretim ve Sınav Yönetmeliği” nin ilgili maddeleri uyarınca kabul edilmiş ve Enstitü Yönetim Kurulu'nun .....tarih ve .....sayılı kararı ile onaylanmıştır.

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ETHICAL DECLARATION

I hereby declare that this thesis is my own work, that I have not acted unethically at all stages from the planning of the thesis to the writing of the thesis, that I have obtained all the information in this thesis within academic and ethical rules, that I have cited sources for all information and comments that were not obtained in this thesis, and that I have included these sources in the list of references, and that I have not violated patent and copyright rights during the study and writing of this thesis.

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LIST OF ABBREVIATIONS

OS	: Oxidative Stress
CRP	: C-Reactive Protein
NF-κB	: Nuclear Factor Kappa B
WBC	: White Blood Cell
COX-1	: Cyclooxygenase-1
HIV	: Human Immunodeficiency Virus
UTI	: Urinary Tract Infections
PMNs	: Polymorphonuclear Neutrophils
COX-2	: Cyclooxygenase-2
NSAIDs	: Non-Steroidal Anti-Inflammatory Drugs
PG	: Prostaglandins
EOs	: Essential Oils
DO	: *Daphne oleoides* subsp. *oleoide*
TL	: *Thymus longicaulis* subsp. *longicaulis* var. *Subisophyllus*
CVS	: Cardiovascular System
AD	: Alzheimer Disease
PD	: Parkinson's Disease
ALS	: Amyotrophic Lateral Sclerosis
MI	: Myocardial Infarction
MIC	: Minimum Inhibitory Concentration
NO	: Nitric Oxide
OR	: Oral Route
PF	: Pulmonary Fibrosis
IC	: Inhibitory Concentration
P.O.	: Per Oral
I.P.	: Interplanar
I.G.	: Intragastric
Met-HCL	: Metformin Hydrochloride
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
NADPH	: Nicotinamide Adenine Dinucleotide Phosphate
INDO	: Indomethacin
TM	: Traditional Medicine
CNS	: Central Nervous System
CH_2CL_2	: Dichloromethane

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1. ÖZET

Tezin başlığı: Türk Geleneksel Tıbbi Bitkilerin Akut İnflamasyon Üzerindeki Koruyucu Etkilerinin İn-vivo Araştırılması

Öğrencinin Adı Soyadı: Abdul Hamid

Danışmanın Adı Soyadı: Prof.Dr. Hatice Kübra ELÇİOĞLU

Programın Adı: Farmakoloji Yüksek Lisans Programı

Amaç: Bu çalışmanın amacı, *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* (TL) ve *Daphne oleoides* subsp. *oleoides'*in (DO) Sprague Dawley sıçanlarında karragenan kaynaklı pençe ödemi ile akut enflamasyon üzerindeki farmakolojik özelliklerini araştırmaktır. Bunun da ötesinde, bu çalışma bahsi geçen iki bitkiye ilişkin asılsız bilimsel faktörleri ortaya çıkarmaya da odaklanmaktadır. Ayrıca, çalışma gelecekteki çalışmalar için bir literatür taraması katkısı sağlamayı da amaçlamaktadır. Aynı zamanda, iki bitkinin deneylerinin değerlendirilmesiyle elde edilen sonuçlar, ileride yeni deneylerin yapılmasına yardımcı olacaktır.

Gereç ve Yöntem: Bu çalışmada akut enflamasyon testleri için otuz tane Sprague Dawley sıçan kullanılmıştır. Karragenan, pençe ödemi oluşturmak için uygulanmış ve indometasin ilacı standart ilaç olarak kullanılmıştır. *Thymus longicaulis* ve *Daphne oleoides*'nun kloroform, etil asetat petrol eteri ve metanol ekstraktları sırasıyla maserasyon yöntemleriyle elde edilmiştir. Sıçanlar, her grup altı sıçan içerecek şekilde beş gruba ayrılmıştır. Ödem gelişiminin hacmi ve süresi pletismometre adı verilen dijital bir makine kullanılarak belirlenmiştir.

Bulgular: Deneylerden elde edilen sonuçlara bakıldığında, *Thymus longicaulis* ve *Daphne oleoides* bitki ekstresi akut enflamasyon üzerinde koruyucu bir etki göstermektedir. TL ekstresi, standart ilaç indometasin ile karşılaştırıldığında DO ekstresi ile karşılaştırıldığında ödem boyutunu yaklaşık %10 oranında düşürmede her saat belirgin bir fark göstermiştir.

Sonuç: Testlerden elde edilen verilerin analiz edilmesiyle, *Thymus longicaulis* bitki ekstresi, *Daphne oleoides* bitki ekstresi ile karşılaştırıldığında akut enflamasyonun korunmasında önemli bir etki ($p<0.001$) göstermiştir. Bununla birlikte, bitkilerin farklı kısımlarının çeşitli çözücülerle araştırılması ve spesifik farmakolojik etkilerden sorumlu fitokimyasal bileşiklerin incelenmesi konusunda hala eksiklikler bulunmaktadır. Ancak klinik deneylerde kullanıma sunulabilmesi için ekstraktların etki mekanizmasının, dozaj formlarının ve toksisite çalışmasının belirlenmesi amacıyla hayvan modelleriyle daha fazla incelemeye ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Pençe Ödemi, İnflamasyon, *Daphne oleoides*, *Thymus longicaulis*

2. SUMMARY

Title of Thesis: In-vivo Investigation of the Protective Effects of Turkish Traditional Medicinal Plants on Acute Inflammation

Student Name, Surname: Abdul Hamid

Supervisor Name: Prof.Dr. Hatice Kübra ELÇİOĞLU

Program Name: Pharmacology Master Program

Objective: The purpose of this exploration is to investigate the pharmacological properties of *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* (TL) and *Daphne oleoides* subsp. *oleoides* (DO) on acute inflammation in Sprague Dawley rats with carrageenan-induced paw edema. On top of that, this work also focuses on revealing the unfounded scientific factors regarding the two plants mentioned. Moreover, the study also aims to provide a literature review contribution for additional processing. At the same time, the results gained by evaluating the assays of the two plants will assist in carrying out new experiments in the upcoming.

Materials and Methods: This study used thirty Sprague Dawley rats for the acute inflammation tests. The carrageenan was applied to induce paw edema, and the indomethacin drug was used as a standard medicine. Chloroform, ethyl acetate, petroleum ether, and methanol extracts of *Thymus longicaulis* and *Daphne oleoides* are acquired by maceration procedures. The rats were categorized into five groupings of six rats each. The volume of edema enlargement was assessed with an electronic machine termed a plethysmometer.

Result: By observing the results obtained from the experiments, the plant extracts of *Thymus longicaulis* and *Daphne oleoides* exhibited a protective effect on acute inflammation. When compared with the standard drug indomethacin, TL extract showed a remarkable variation at each hour in reducing the dimension of edema by 10% more or less than DO extract.

Conclusion: By analyzing data acquired from the tests, the *Thymus longicaulis* plant extract showed a significant effect ($p<0.001$) on the protection of acute inflammation than the plant extract of *Daphne oleoides*. However, there are still gaps in investigating the different parts of the plants with various solvents and examining the responsible phytochemical compounds for the specific pharmacological effects. Furthermore, supplementary investigational trials with animal models will be necessary to ascertain the mechanism of action, dosage forms, and toxicology screening of the extracts before they can be recommended for further clinical trials.

Keywords: Paw Edema, Inflammation, *Daphne oleoides*, *Thymus longicaulis*

3. INTRODUCTİON and PURPOSE

Since antiquity, humans (Homo sapiens) have been aware that several plants around the world have medicinal characteristics and can be used to treat various ailments (Abdallah et al., 2023). Humans have been using medicinal plants to treat or manage various types of diseases for a long time. The oldest record of the utilization of curative plants for health has been identified on a Sumerian clay slab from Nagpur, which is almost 5000 years old. Additional evidence was observed in Egypt, Mesopotamia, Islamic civilizations, and Greece. For the first time, the utilization of nearly 1000 plants was documentary as medicinal plants (Süntar, 2020). Numerous ethnopharmacological studies have demonstrated that approximately two-thirds of the medicinal species around the globe have significant antioxidant properties. Moreover, they are incredibly essential and advantageous for lowering oxidative stress (OS) in cellular regions of the body and act as a vital responsibility in treating and managing several diseases, including cardiovascular diseases, inflammatory disorders, hepatic issues, and cancerous problems (Zafar et al., 2023). Ethnobotanical research has exhibited that remedial plants have been enhanced to be used as historic and modern treatments for treating different diseases. The plants should be used for the treatment accurately, precisely, and according to the physicians' guidance to avoid harmful side effects that could be fatal (Abduraimov et al., 2023). Archaeologists showed that the relationship between plants and humans is approximately 1.2 million years old. Recent research has illustrated that plants provide 70-80% of daily calories. Nowadays, the consumption of medicinal substances has dramatically increased due to the bio-adverse effects of pharmaceuticals, and it is a fact that 80% of the world's indigenous population uses them against various medical diseases, and 80,000 medicinal species are currently used for therapeutic purposes (Karahan, 2023).

Inflammation can be identified by observing the following five signs: puffiness, tenderness, heat, redness, and destruction of cellular activity. Inflammation was also defined as the changes that occur in a living tissue when it is damaged by an external factor, such that the damaged tissue is neither destroyed nor viable. However, the ancient people recognized inflammation as a healing process and the body’s defensive response until the 19th century. After the 19th century, inflammation was marked as an undesirable reaction, hazardous to the host (Punchard et al., 2004). Most pathologists consider inflammation to be a pathophysiological response to living tissue injuries, and it is a fundamental way of the body's defense system's response to infection, irritation, and other types of wounds and injuries (Stankov, 2012). The level of stress can manipulate the stage of the inflammation mechanism, where chronic stress suppresses immune function, acute stressors stimulate the immune response, and heavy stress highly activates the immune system, primarily leading to abnormal anti-inflammation and inflammation.

Pharmacological studies from different sources reported that pro-inflammation is induced by various inflammatory arbitrators such as the transcription factor of nuclear factor kappa B (NF-κB), IL-6, TNFα, IL-1β, and C-reactive protein (CRP). Additionally, neuroinflammation (central and peripheral inflammation) is identified due to stressful situations (Liu et al., 2017). The prominent nuclear factor kappa B (NF-κB) pathway is considered a remarkable part of the body's inflammatory response. This is because it assists in controlling the production of proinflammatory molecules like cytokines, chemokines, and adhesion proteins, which play prominent roles in inflammation (Lawrence, 2009). Inflammation is a vital physiological protective response of the body's immune system to harmful stimuli such as infections, tissue injury, or other noxious scenarios. It performs a crucial function in eliminating harmful agents, advancing healing, and sustaining tissue homeostasis. As a component of inherited human immunity, it serves as the first line of defense by detecting molecular recognition patterns linked to acute inflammation, infection, or injury. This sophisticated mechanism encompasses a selective articulation of certain key regulators and pro-inflammatory molecules. However, elongated or dysregulated inflammation, often influenced by persistent stimuli or genetic factors, can lead to inflammatory diseases and other pathological conditions, posing significant health challenges (Ahmed, 2011). The inflammatory response is a significant mechanism by which tissues respond to damaging factors involving primary defensive cells such as macrophages, neutrophils, and lymphocytes (WBC). These cells circulate mediators, including vasoactive amines, eicosanoids, pro-inflammatory cytokines, and anti-acute plasma proteins, to regulate inflammation, protect against further damage, and facilitate tissue repair. However, dysregulated inflammation can progress to chronic states, contributing to the promotion of diseases such as the occurrence of cancer, kidney problems, cardiovascular disorders, heart diseases, pulmonary disorders, and neurodegenerative conditions (Abdulkhaleq et al., 2018).

Non-steroidal anti-inflammatory drugs (NSAIDs) are broadly utilized globally for their anti-inflammatory, antipyretic, and analgesic effects. However, their therapeutic utilization is frequently associated with gastrointestinal side effects due to the non-selective inhibition of cyclooxygenase (COX)-1 and COX-2 enzymes, which decreases the gastroprotective prostaglandins (PG) hormone. NSAIDs are assumed to be accountable for approximately 5–10% of global prescriptions, with an estimated 30 million individuals consuming them daily. Usage is particularly prevalent among older populations, with 96% of patients aged 65 and above reporting NSAID use and 7.3% of individuals over 60 filling at least one NSAID prescription annually (Sohail et al., 2023). Indomethacin, a repeatedly prescribed NSAID, inhibits prostaglandin (PG) biosynthesis via cyclooxygenase-2 (COX-2), providing anti-inflammatory, analgesic, antipyretic, and anti-aggregatory effects. It is commonly used to

manage inflammation, pain, rheumatic diseases, and acute gout attacks (Madkhali et al., 2023). In our evaluation, indomethacin was used as a reference medicine to compare with the control and disease groups.

In the current research, the extracts of the aerial part of *Daphne oleoides subsp. oleoides* are conducted for the investigation of pharmacological properties. Petroleum ether, chloroform, and methanol extracts are acquired consecutively by the maceration approach. The acute inflammation effects are examined by utilizing the paw edema model techniques induced by carrageenan. The researchers have evaluated that the *Daphne* species possess a broad range of phytochemical compounds, including coumarins, sesquiterpenes, flavonoids, lignans, diterpenes, steroids, and triterpenes. Numerous biological properties of *Daphne* species were found, including anti-inflammatory, hemostatic, anti-cancerogenic, antiulcerogenic, antioxidant, analgesic, antimicrobial, hypocholesterolemic, and abortive (Süntar et al., 2012). *D. oleoides* was investigated for its potential protection against pulmonary fibrosis in bleomycin-induced rats by lowering antioxidant and inflammatory markers (Danaei et al., 2023). *Daphne oleoides* has the pharmacological properties of antimicrobial, antifungal, cytotoxic, and hypoglycemic activity (Muddassir et al., 2022). The *Daphne* genus, an element of the *Thymelaeaceae* family, encompasses 70 species distributed across Europe, the Philippines, subtropical and clement regions of Asia, North Africa, Australia, and the Pacific (Riaz et al., 2016). However, several investigations have been carried out on the *Daphne oleoides* species for different medicinal activities, there is still a gap in clinical research to evaluate the exact dose and dosage forms for certain ailments. Hence, this study assessed the pharmacological impact of *Daphne oleoides* on acute inflammation in Sprague Dawley rats' paws by inducing carrageenan in an *in vivo* experiment.

The genus *Thymus* is a member of the family of Lamiaceae, which contains approximately 214 species and 36 subspecies throughout the globe. The plants are mainly found around the Mediterranean Sea, basically the Iberian Peninsula, Europe, Turkey, Greenland, North America, Northwest Africa, Abyssinia, and Asian countries (Li et al., 2019). The phytochemical exploration has exhibited that essential oils (EOs) present in the *Thymus* species are mainly comprised of thymol, carvacrol, esters, terpenes, phenolic derivatives, and terpene alcohols. Other phytochemical compounds found in these species are flavonoids, lignans, organic acids, simple phenylpropanoids, tannins, phytosterols, and terpenoids. Pharmacological research has demonstrated that the biochemically active compounds and extracts of the *Thymus* species have a broad range of therapeutic properties in vivo and in vitro evaluation, such as antioxidant, antitumor, anti-inflammatory, analgesic, antimicrobial, antitussive, antihypertensive, antispasmodic, anthelmintic, antidiabetic, and carminative etc.

Thymus genus plants are renowned for their distinctive utilizations worldwide, particularly in the cosmetic, food, traditional treatment, pharmaceutical, and perfumery industries. *T.vulgaris* L. (common thyme) is a significant and unique species and has been widely used for a long time in history among the species (Salehi et al., 2019). Nevertheless, other species such as *T. zygis* L. (Spain thyme), *T. pulegioides* L. (large thyme), and *T. serpyllum* L. (wild thyme) are also broadly utilized for commercial aspects around the world (Stahl-Biskup & Venskutonis, 2012). Ethnopharmacological investigations have revealed that the three Thymus L. species, explicitly *Thymus pubescens*, *Thymus praceox* subsp. *grossheimii* and *Thymus cariensis* possess the pharmacological activities of antioxidants, anticholinesterase, anti-urease, and anti-tyrosinase (Eroglu Ozkan et al., 2022). A study showed that *Thymus longicaulis* subsp. *longicaulis* var. *longicaulis* contains the compounds of the oil of thymol, p-cymene, and γ - terpinene and antioxidant effects (Sarikurkcu et al., 2010). *Thymus longicaulis* C. Presl. (Lamiaceae) is a minuscule aromatic herb plant mostly found in the Illyric-Mediterranean region, used traditionally as a treatment for cold, abdominal pain, flu, nephritis, and cough, and presents antioxidant and anti-inflammatory actions (Galasso et al., 2014). Studies have explored that essential oils and flavonoids of the *Thymus* possess therapeutic activities, including antiseptic, antimycotic, expectorant, antibacterial, antioxidant, spasmolytic, and food preservatives. Taxa is one of the species of the *Thymus*, termed “kekik” in Turkish, and is consumed as herbal tea, spice, and for healing reasons for infection (Elkıran, 2022).

4. GENERAL INFORMATION

4.1. Daphne genus

The Daphne genus (*Thymelaeaceae*) naturally occurs as small-sized shrubby plants mostly located in tropical and sub-tropical environments around the globe (Banerjee et al., 2022). The Daphne genus is one of the most commonly found species, and it belongs to the family of *Thymelaeaceae*. The *Thymelaeaceae* contains more than 90 species, and they are especially found in Europe, Asia, and some areas of North Africa. The Daphne species are extensively employed in Chinese traditional medicine for the therapy and management of miscellaneous disorders. The species of the Daphne genus are being utilized to treat a multiplicity of diseases, such as diarrhea, abscesses, and rheumatism (Sanda et al., 2015). The species of Daphne possess various pharmacological properties, including antioxidant, antiviral, antibacterial, anti-inflammatory, antifungal, analgesic, and abortive activity (Moshiashvili et al., 2020). Various phytochemical research on the Daphne genus has been conducted, and many biologically active compounds, including lignans (Zhang et al., 2018), flavonoids (Du et al., 2016), and diterpenoids (Zhan et al., 2005), have been identified. All these phytochemical compounds are assumed to have pharmacological effects on tumors, HIV, inflammation, and neurological diseases (Hou et al., 2021). The daphne species also contain other essential chemical compounds such as betulin, simarin, and flavon, and are responsible for anti-gout, anti-cancer, anti-microbial, and anti-inflammation activities (Khodayari et al., 2017). The plants of the Daphne genus have plenty of natural diterpenoids, and approximately 135 diterpenoid compounds have been isolated so far. Numerous ethnopharmacological studies in vitro and in vivo have exhibited that these diterpenoid biological compounds are responsible for the effects of anti-HIV, anti-inflammatory, anticancer, antifertility, and antihyperlipidemic (Nie et al., 2021).

4.1.1. Taxonomical background of *Daphne oleoides*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Malvales
Family	Thymelaeaceae
Genus	*Daphne* Tourn. Ex L.
Species	*Daphne oleoides* Schreb.
Sub-species	*Daphne oleoides subsp. oleoide*

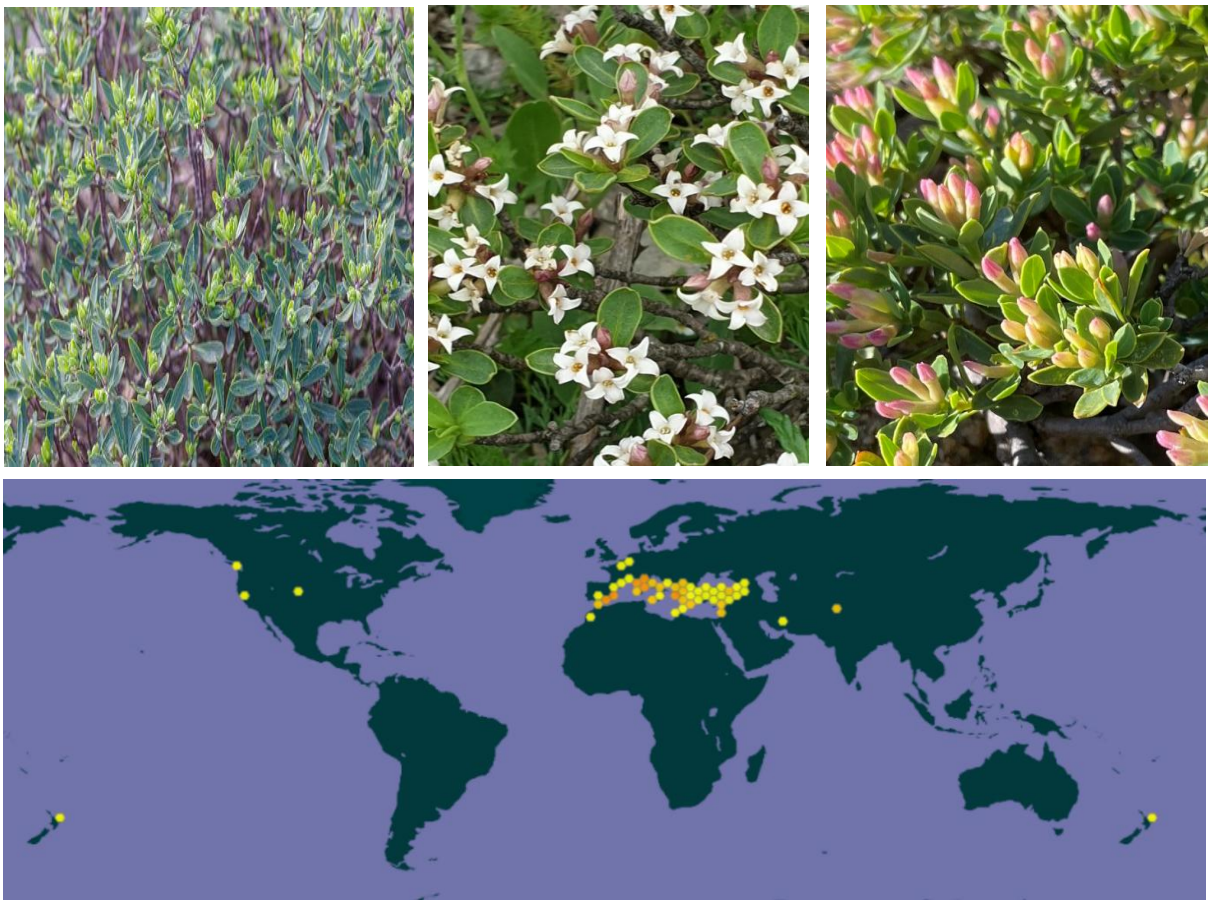


Figure 1. *Daphne oleoides* plant

4.1.2. Distribution and Description of *Daphne oleoides*

The *Daphne* genus, an element of the *Thymelaeaceae* family, encompasses 44 families with nearly 500 herbal species and is distributed across Europe, the Philippines Islands, subtropical and temperate regions of Asia, Australia, North Africa, Turkey, and the Pacific (Miroslav et al., 2016; Riaz et al., 2016). The *Daphne oleoides* is a small green shrubby plant, and it generally grows connected to calcareous rocks at 300 – 2300 m altitude. The plant is heavily branched up to 70 cm in height, together with persistent leaves and leathery. The flower smells aromatic and perfumed, and they are purple outside, and white inside, and seeds have occurred at the end of the summer. The flowers are generally 10 – 15 mm long and linked into 2 – 6 terminal heads. The Daphne genus has been noticed for its significant and valuable medicinal compounds and many species, including *D. genkwa* and *D. oleoides* are widely applied as conventional medicine and ethnomedicine for the procurement of cancers, aches, edemas, inflammation, malaria, ulcers, gonorrhea, high blood pressure, fever, rheumatism, and microbial infection (Uysal et al., 2017; Venditti et al., 2017; Venditti et al., 2019).

4.2. Thymus genus

Lamiaceae is a mint and aromatic family with 220 genus groups and more than 4000 species worldwide. It is one of the most prominent and common families of blooming plants worldwide. The genus *Thymus* L. is a part of the Lamiaceae and subfamily Nepetoideae, consisting of approximately 215 species. It is a smaller size, shrubby, perennial, and herbaceous plant extensively dispersed in the Mediterranean region (Jamzad, 2010). Some florae of this presented in Iran are such as *T. eriocalyx, T. kotschyanus, T. migricus, T. pubesens, T. fallax, T. trautvetteri, T. transcaspicus, T. persicus, T. nummularius, T. caucasicus, T. daenensis,* and *T. fedtschenkoi.* The herbs of the plants in this genus have been broadly practiced in traditional medical treatment for many diseases for a long time, and are also applied as food preservatives and in pharmaceutical manufacturing. The plant is also utilized as a remedy to treat artery problems, UTI (urinary tract infections), toothache, and respiratory diseases such as whooping cough, asthma, and bronchitis (Hashim & Gamil, 1988). The essential oils of this genus are such as flavonoids, thymol, saponins, luteolin, tetra methoxylated flavones, eugenol, aliphatic phenols, and carvacrol, and they have the pharmacological properties of antibacterial, antifungal, antiseptic, antioxidant, and antiparasitic (Ghasemi Pirbalouti et al., 2015). Another ethnobotanical review study has demonstrated that the genus possesses important volatile oils, including tannins, terpenoids, phytosterols, flavonoids, organic acids, phenylpropanoids, and pharmacotherapeutic activity of antidiabetic, cytotoxic, anti-inflammatory, analgesic, antimicrobial, and antioxidant (Li et al., 2019). The genus is also famous for its distinctive features, including significant medical properties such as antihypertensive, antibacterial, antitumor, antipyretic, anticancer, antiviral, anti-inflammatory, antifungal, antioxidant, and biochemical compounds, including alkaloids, flavonoids, phenolic acids, carvacrol, thymol, *p*-cymene, terpenoid, and geraniol (Anwar et al., 2024).

4.2.1. Taxonomical background of *Thymus longicaulis*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Thymus* L.
Species	*Thymus longicaulis* C. Presl
Sub-species	*Thymus longicaulis subsp. longicaulis var. Subisophyllus*



Figure 2. *Thymus longicaulis* plant

4.2.2. Distribution and Description of *Thymus longicaulis*

Thymus longicaulis was discovered by Carl Borivoj in 'Flora Sicula' during a trip in 1817. The plant has creepy branches, sometimes ending with an inflorescence, and the stems are 4-16 cm long and hairy. The leaves are herbaceous, and the base of the leaves is covered with sessile glands; they are generally red and sometimes yellow. The plants flower from April to July commonly. The plant is mainly inhabitant to the Mediterranean zone and widely distributed in the Italian Peninsula, southern Austria, Turkey (European side), Slovenia, the Balkan Peninsula, and Anatolia (Bartolucci & Domina, 2014). *Thymus longicaulis subsp. longicaulis var. Subisophyllus* is a tiny, aromatic, shrubby, perennial herb plant that mainly grows in the Mediterranean region, and it is named 'timo a fascetti' in Italy. *Thymus* plants are generally called 'kekik' in Turkish and are frequently consumed as spice, herbal tea, and medicinal species (Baytop, 1999). It grows widely in acidic soil at a height ranging between 600-1950 m a.s.l (Campolo et al., 2016). TL is a famous traditional medicinal plant that treats numerous diseases like cough, fever, inflammation, abdominal pain, and flu. The plant also possesses strong anti-inflammatory and antioxidant properties (Galasso et al., 2014).

4.3. History of Traditional Medicinal Plants

Humans have been using medicinal herbs as a main origin of therapeutic remedies for many decades to cure various diseases. Many ailments are treatable by using crude drugs that have been descended from traditional botanical plants, including metformin, used to cure diabetes. However, new drugs have been discovered applying medicinal chemistry, which could be useful widely for managing newly found diseases. Numerous phytochemical compounds that are of plant origin are considered to be therapeutically effective in treating illnesses such as diabetes, inflammation, and neurological and immunological disorders. This type of treatment showed minimum side effects and affordable cost in several scenarios compared to synthetic or semi-synthetic medicine (Laurindo et al., 2023). Traditional medicine is a broad science and has a significant relationship with natural products based on their utilization. Microorganisms, medicinal plants, animals, and minerals are used in traditional medicine (TM) and include various types of practices such as Kampo, Ayurveda, Unani, and Homeopathy. These practices have been applied to treat many diseases and prevent symptoms of various disorders around the world (Yuan et al., 2016).

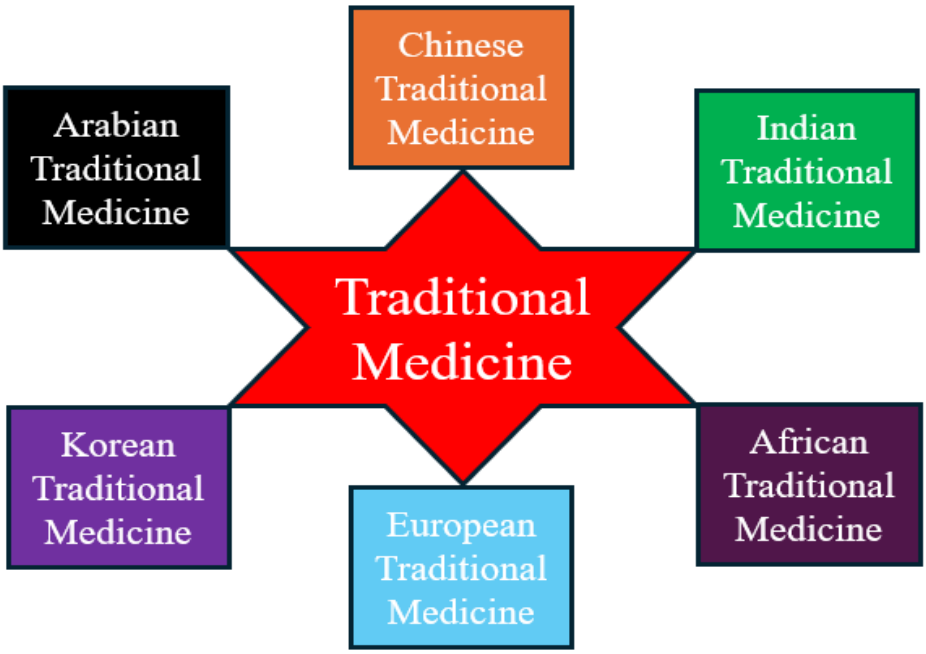


Figure 3. Types of traditional medicine around the globe (Sophia et al., 2023)

Traditional medicinal plants have become a crucial source for healthcare systems in most undeveloped countries, particularly for those living in rural and suburban regions. Nearly 60% of the population of some African countries have been using traditional remedies to cure and manage acute and chronic illnesses and infectious diseases, psychological problems, and mental disorders as well (Sitoe & Van Wyk, 2024). The prominent utilization of medicinal

plants had been considered for the treatment of diseases rather than prevention of the disorders and traditional therapeutic management of ailments contained approximately 90 % of the usage of medicinal plants. Pharmacological research has been conducted globally to investigate the efficacy and potency of medicinal plants for the treatment of diseases and some scientific outcomes have supported to production of medicine in the pharmaceutical industries based on medicinal plants. The pharmaceutical industries manufacture medicine of the value of 100 USD billion per year by using medicinal plants. Medicinal plants could be an essential part of the prevention and protection of diseases and their usefulness in the prevention of illnesses is investigated under the approach of the five Primary Health Care main principles (Sofowora et al., 2013).

The world population has been using medicinal plant-based treatment since time immemorial, and they are also the prime source for the manufacturing of modern allopathic medicines, pharmaceuticals, and food supplements, including vitamins (Khumalo et al., 2022). Medicinal herbs possess a richness of components of secondary metabolic compounds and bioactive products that dominate primary factors in the healing impact of several infections and illnesses. Perhaps many medicinal plants restore the body's immune system naturally and biologically maintain the homeostatic condition (Taylor et al., 2001). The interest in pharmacological activities of traditional medicinal plants has been dramatically enhanced recently because of the rising problems related to the use of allopathic products, bacterial resistance development of synthetic and semi-synthetic medicine, and their decreasing efficacy gradually and unmanageable side effects (Van Vuuren & Muhlarhi, 2017) (Ong et al., 2007) (Rao & Knaus, 2008). It is noticed that approximately 50% of globally used pharmaceutical medicines are originally obtained from phytochemical products (Balandrin et al., 1993). Quinine is an important exemplary medicine gained from plant sources used to treat malaria, isolated phytochemically from the plant bark of the *Cinchona officinalis* L. plant (Misra et al., 2008). Since 400 BC, medicinal plants that contain the biological compound acetylsalicylic acid have been utilized therapeutically to treat pain, inflammation, and pyrexia (Rao & Knaus, 2008).

4.4. What is inflammation, and the relation of inflammation to other diseases

The term inflammation derives from the Latin word “inflammare, " meaning to burn. The inflammatory response is one of the prime processes that essentially entail the defense system of human cells to protect them from wounds, injuries, and mycobacterial infections (Abdulkhaleq et al., 2018). However, the inflammation normally could be analyzed as acute, chronic, short-term, or long-term (Serhan et al., 2015). Studies have shown that chronic inflammation is detected due to the numerous different complications with other disorders,

including cancers, neurological diseases, and cardiovascular problems (Uttara et al., 2009). The primary pathophysiological mechanism of inflammation is the main pathophysiological resistance of viable tissue to wounding, which evolves through the localized aggregation of plasma fluid and blood components. It is a protective response of the body purposed at eliminating or hampering the dissemination of a harmful agent. This reaction is identified by five fundamental indicators: heat, redness, pain, swelling, and impaired function (Hossain et al., 2012).

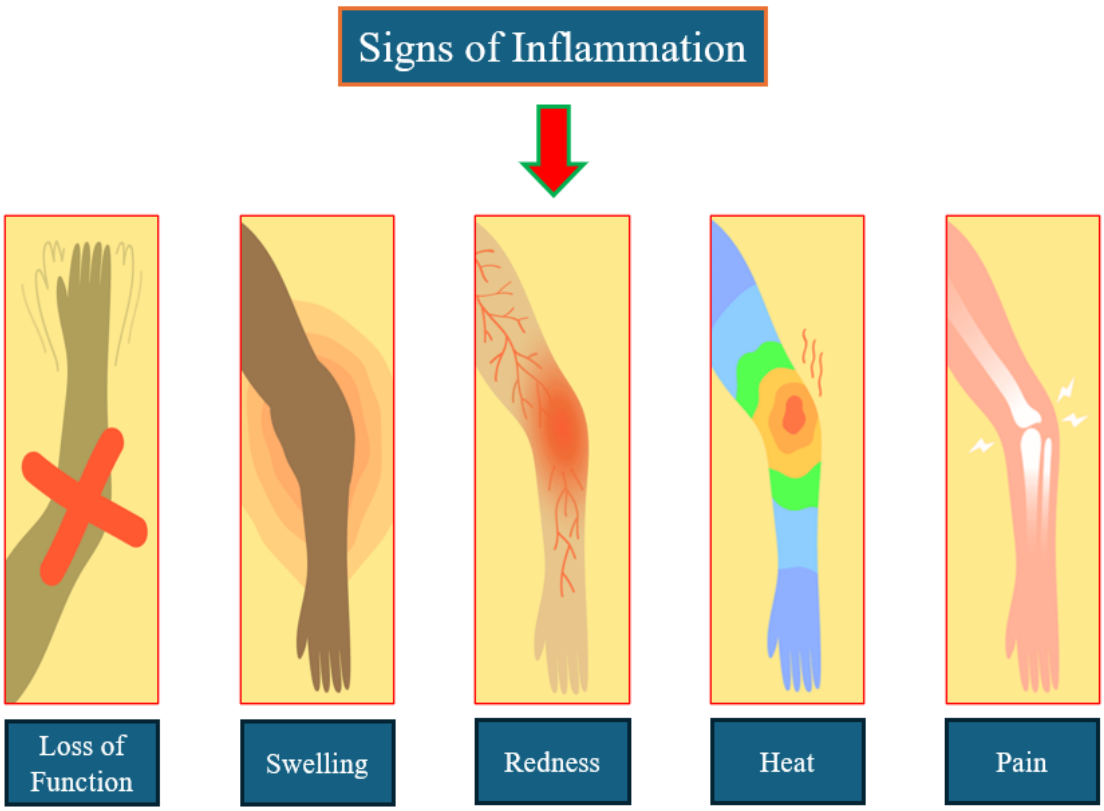


Figure 4. Five basic signs of inflammation (Scott et al., 2004)

The immune system functions by reacting to the presence and problematic pathogens within the body. Inflammatory mediators, among them cytokines, PG, histamine, and leukotrienes, are conveyed by leukocytes or inflamed tissues. The inflammatory mediators' compounds will account for the inflammation (Rousdy et al., 2022).

Pharmacological research has shown that inflammation has been related to various types of pathogenic diseases, including diabetes, infectious diseases, asthma, COPD, arthritis, and malignant tumors. In inflammation, the crucial involvement of polymorphonuclear neutrophils (PMNs) proceeds from their movement into the tissue of the inflammation area, response to chemokines, and their capacity to eradicate pathogens through actions such as phagocytosis, degranulation, the formation of neutrophil extracellular traps, and the release of cytokines. The release of endogenous reactive oxygen species by NADPH-oxidase actions maintains pathways

relying on tyrosine phosphorylation to regulate the cytokines and chemokines expression involved in the inflammation actions. Inflammation is a body's self-defense reaction to injuries and to remove the invaded pathogens and foreign harmful agents from the body. Inflammation also adds contribution to the pathophysiology of the development of many serious chronic diseases. When the inflammatory response becomes weak and unworkable, the body gets infected and diseases. Acute or moderate inflammation is a short reaction, and chronic or unending inflammation response is a long-term feedback. It is related significantly to many serious ailments such as diabetes, obesity, cancer, auto-immune disease, and CVS diseases. One of the best examples of this scenario is atherosclerosis, which is caused by plaque disruption and thrombosis and is also intimately related to inflammation (Chen et al., 2024; Libby, 2007). Chronic inflammation is greatly associated with aging disorders that are caused in older people, such as Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD), Myocardial Infarction (MI), and atherosclerosis (McGEER & McGEER, 2004). The nuclear transcription factor (NF)-κB is the prime mediator of inflammation. Immunoregulatory mediators known as proinflammatory cytokines are produced by immunological cells (such as macrophages and monocytes) and non-immune cells (including fibroblasts and endothelial cells) responding to diverse stimuli. Following the activation of COX-2, TNF-α and IL-1β prompt the generation of PGE2. Furthermore, TNF-α induces iNOS expression, enhances neutrophil motion, and commences nitric oxide (NO) delivery (Bidian et al., 2022). The inflammation triggered by tissue damage, infectious agents, irritation, or intense heat could be acute or chronic, with the evaluation of phases of vascular, cellular, or resolution (Yousseu Nana et al., 2023). Acute inflammation represents the body's first reaction to an infectious pathogen or an inflammatory elicitor, such as tissue damage from irradiation or wounds. Prolonged or chronic inflammation entails a continuous change in the composition of tissues at the site of inflammation, along with concurrent tissue destruction and repair resulting from persistent inflammatory activity (Calder et al., 2013). Exposure to harmful chemicals and toxic environmental agents can cause chronic inflammation, including cigarette smoke (Stone et al., 2018).

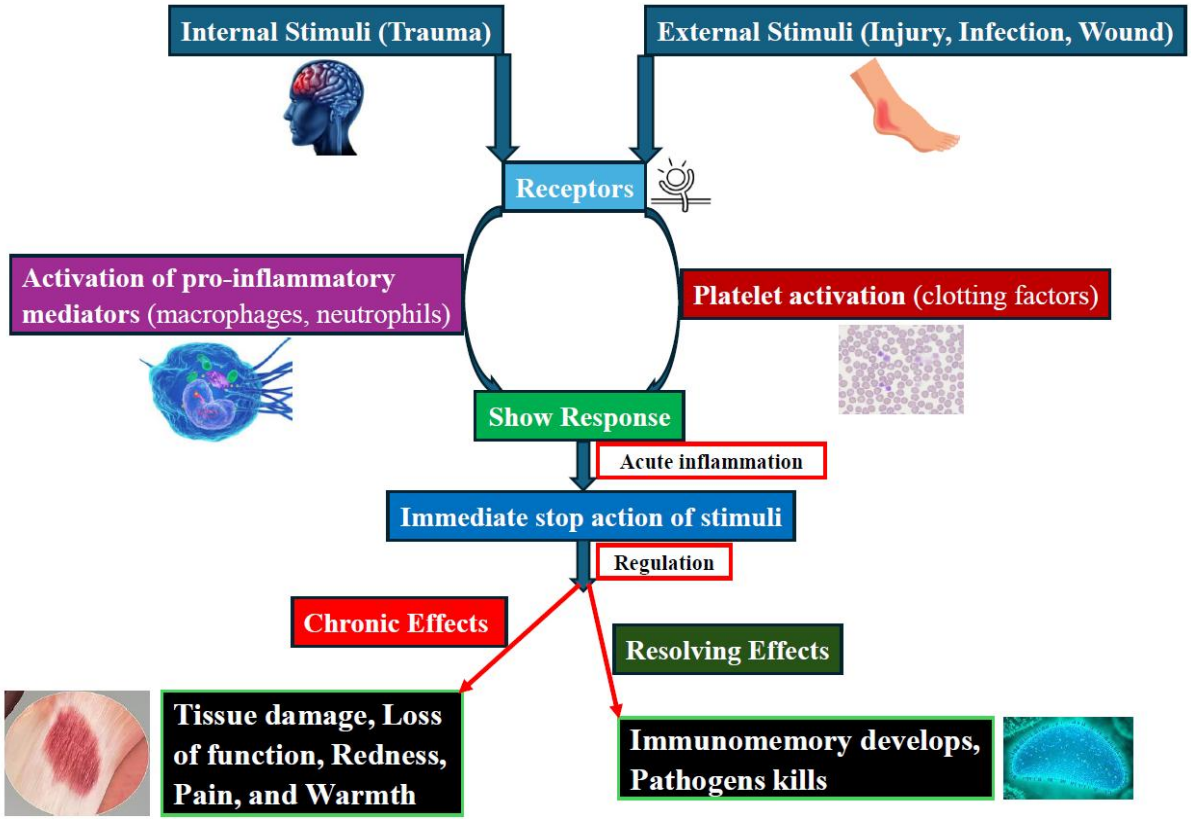


Figure 5. Acute and chronic inflammation in animals (Muzamil et al., 2021)

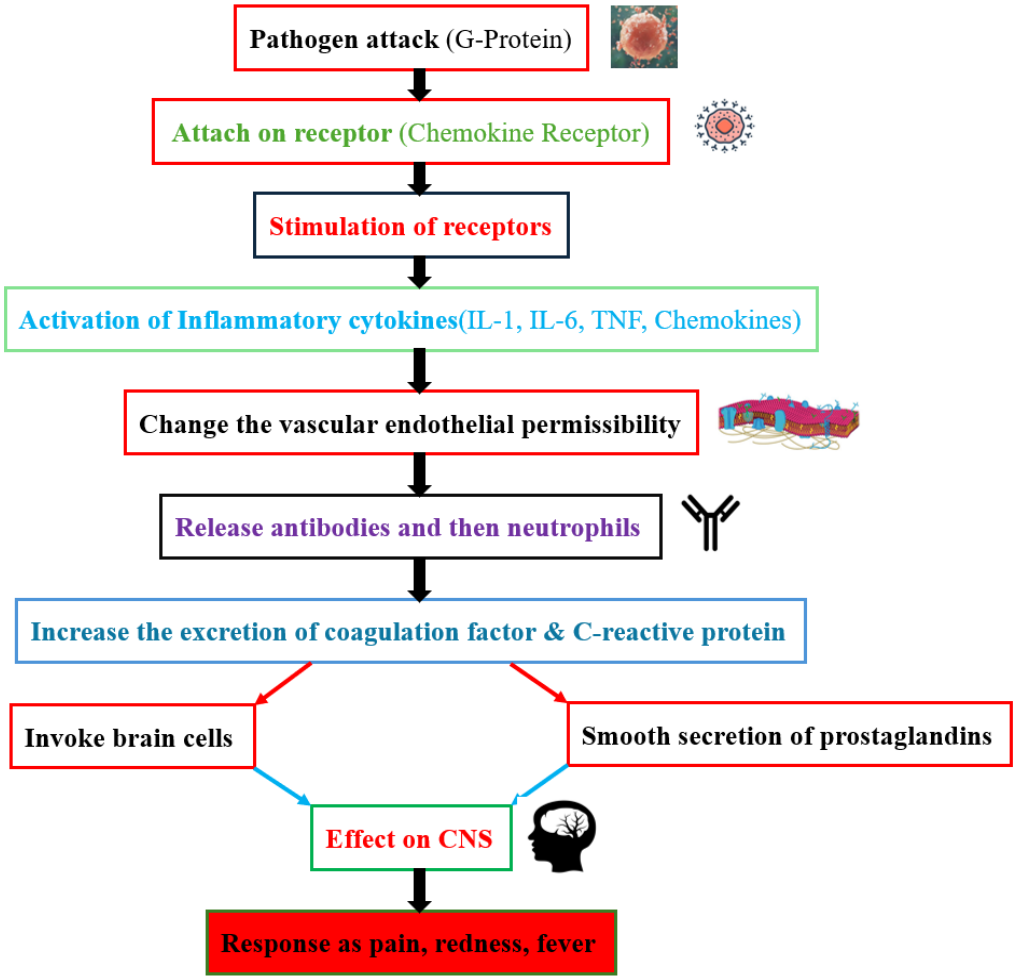


Figure 6. Step of the inflammation process (Muzamil et al., 2021)

4.5. Nonsteroidal Anti-inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a category of medicines that aid in the relief of inflammation. They block the actions of cyclooxygenase (COX) enzymes that produce prostaglandins. The research has found two classes of COX isoenzymes, COX-1 and COX-2. Cyclooxygenase-1 is constitutive and releases PGs that protect the stomach wall from acidity and nephrological injuries. On the other aspect, Cyclooxygenase-2 is responsible for the initiation of inflammation that occurs by stimulation of cytokines and PGs, which produce pain and inflammatory swelling. Therefore, the selective COX-2 antagonists could be the medication for the proper management of inflammation with limited side effects, excluding stomach and kidney problems (Vane & Botting, 1998). A common nonsteroidal anti-inflammatory (NSAID) drug, indomethacin, has the potent effects of analgesia, antipyretic, and anti-inflammatory, and has been impactfully prescribed for the treatment of slight to considerable pain since 1960 (Nalamachu & Wortmann, 2014). NSAIDs, including indomethacin, antagonize the activities of COX-1 and COX-2 enzymes, thereby avoiding the synthesis of thromboxane and prostaglandins. Consequently, they exhibit antipyretic, analgesic, and anti-inflammatory effects (Ou et al., 2019). In ancient times, it was found that plant extracts involved salicylate chemical compounds that could be useful for the management of painful problems, inflammation, and high fever. Nearly one century before the scientist Felix Hoffman discovered aspirin, which inhibits COX-1 and COX-2 actions and lowers the synthesis of mediators of inflammation such as thromboxane and prostaglandins.

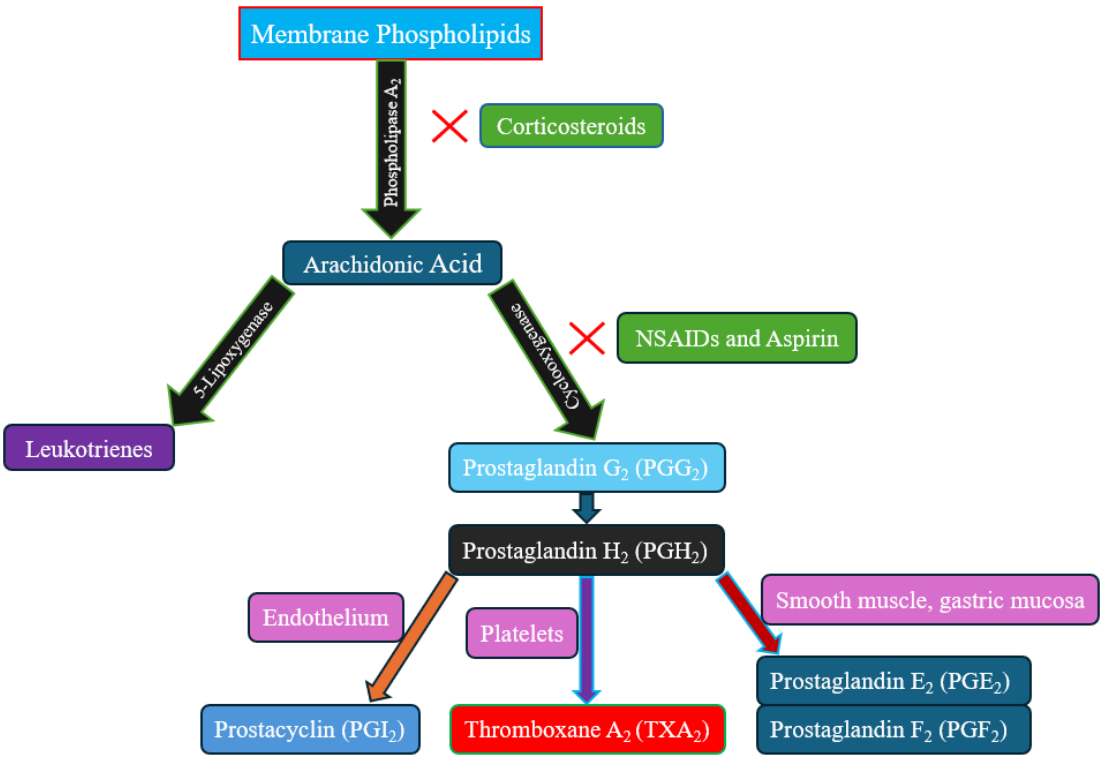


Figure 7. Mechanism of action of NSAID (Sobhani et al., 2023)

Aspirin is one the most useful medicines in the world for its distinctive actions of blocking thromboxane and prostaglandin release (Dinarello, 2010). Additionally, NSAID medicines obstruct the progression of Alzheimer's disorders and selective COX-2 antagonists may exhibit the recent essential therapeutic effect against cancer, Alzheimer's disease progress, and premature labor (Vane & Botting, 1998). Nonsteroidal anti-inflammatory drugs are primarily absorbed in the gastrointestinal pathway and bioavailability is relatively high. Some NSAID drugs such as diclofenac follow first-pass hepatic metabolism resulting in lower bioavailability. There are some NSAID prodrugs (Sulindac sulfide, valdecoxib), which are required to undergo hepatic metabolism to be active metabolites for therapeutic effects. NSAID drugs are maximumly combined with plasma protein and mainly metabolized in the liver and secreted outside with the urine. The half-life of the NSAIDs could be varied among the medicines (Aspirin 0.25-0.3 hours / Piroxicam 45-50 hours) and the weights of body and water present in the patients (Wongrakpanich et al., 2017).

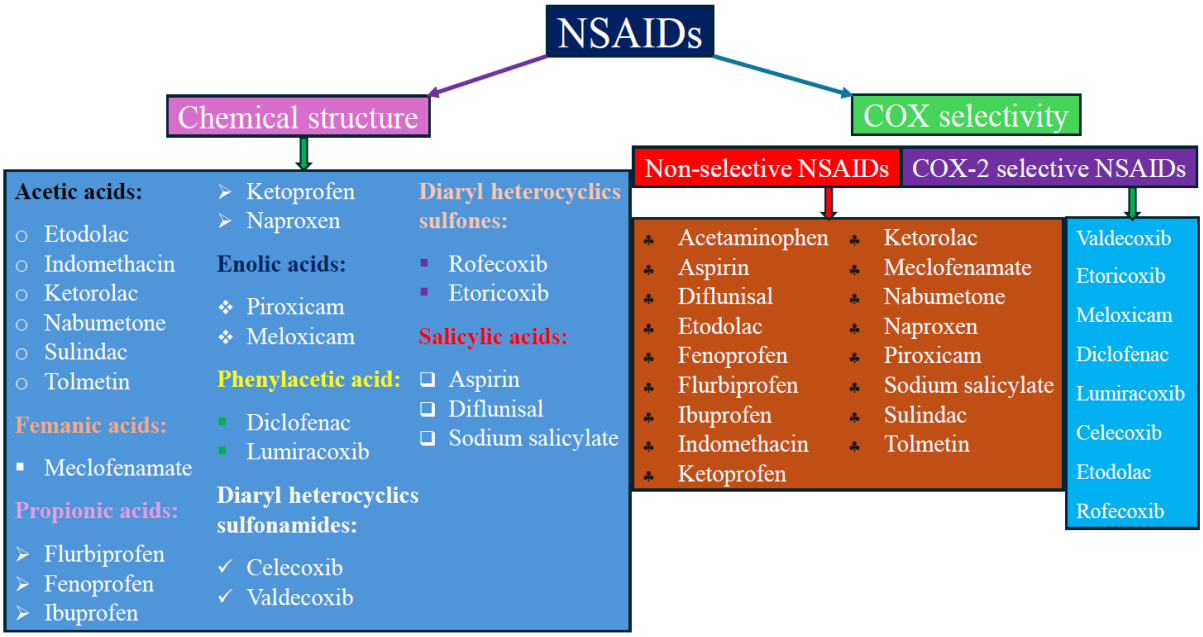


Figure 8. Classification of NSAIDs (Ferreira et al., 2024)

4.6. Indomethacin (Standard Drug)

The novel drug indomethacin is one of the extensively utilized nonsteroidal anti-inflammatory drugs (NSAIDs) around the world, and it is distinctly capable of controlling serious inflammation and pain in several scenarios, together with joint problems and arthritis (Otake et al., 2025). It is a well-known drug of NSAID category with distinctive effects against fever, anti-inflammatory, and analgesic effects. After completing the clinical trials in 1961, the indomethacin drug was available for patients in 1965. The mechanism of action of indomethacin is similar to other NSAID medicines, inhibiting the cyclooxygenase enzyme

(COX) and gradually blocking the release of prostaglandins (PG) from arachidonic acid. There are two sorts of COX enzymes: COX1 and COX2. COX1 is usually found in most of the organs in the body and is responsible for the release of prostaglandins and thromboxane A2. On the other hand, COX2 is accountable for the response to wounds and inflammation. There are also two kinds of NSAID drugs based on the COX enzymes: selective COX1 and selective COX2 inhibitors. Indomethacin, aspirin, ibuprofen, naproxen, and phenylbutazone are popular drugs in the NSAID group. Indomethacin is quickly absorbed from the oral route (OR) and has approximately 100% bioavailability at its peak level. The absorption rate of indomethacin depends on the existence of food in the stomach. The present food in the stomach decreases the rate of indomethacin absorption but not the value of bioavailability.

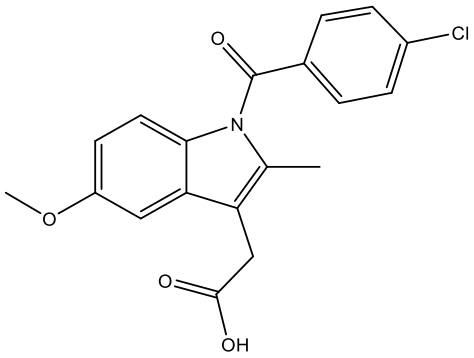


Figure 9. Structure of the indomethacin drug

The mechanism of action of indomethacin is drawn from the research on the pharmacology of indomethacin.

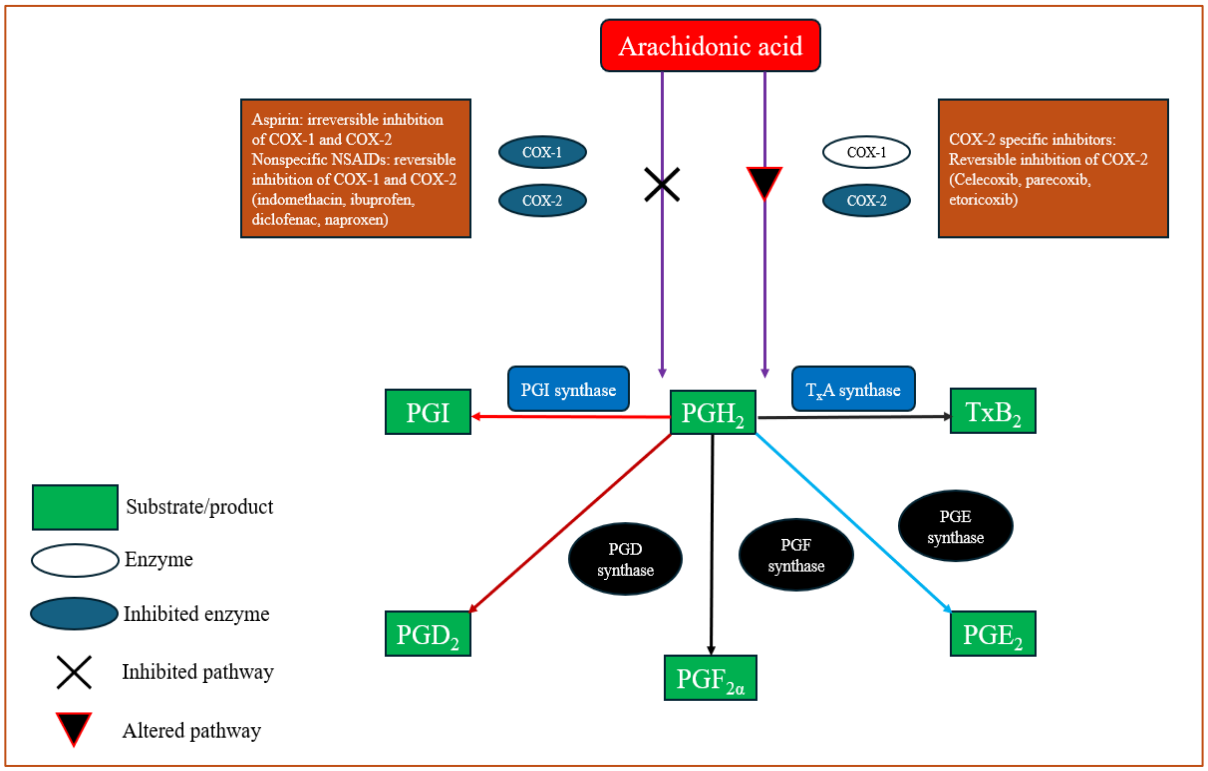


Figure 10. Mechanism of action of indomethacin medicine (Lucas, 2016)

Table 1. Traditional medicinal uses of *Daphne oleoides subsp. oleoide*

Serial Number	Plant parts	Traditional uses	Country of uses	References
1	Aerial part	Rheumatoid arthritis, antipyretic, and lumbago	Turkey	(Yeşilada et al., 2001)
2	Meshed roots	Used to treat malaria, fever, wounds, lumbago, and rheumatism	Turkey and Pakistan	(Riaz et al., 2016)
3	Aerial part	Used to heal wounds, rheumatism, and malaria.	Turkey	(Süntar et al., 2012)
4	DO powdered root combination with sugar	Used to cure malaria	Turkey	(Fujita et al., 1995)
5	An ointment prepared from the aerial part of DO, made up of butter	Utilized to manage rheumatic pain and wound healing.	Turkey	(Yeşilada et al., 1995)
6	Roots of DO	Used as laxative agents.	Turkey	(Moshiashvili et al., 2020)
7	Cortex and leaves of DO	Used to treat skin damage and ulcers.	Turkey	(Moshiashvili et al., 2020)
8	Above ground part of DO	Used to cure fever, lumbago, and rheumatic pain.	Turkey	(Moshiashvili et al., 2020)
9	Leaves of DO	Utilized to treat gonorrhea and abscess.	Turkey	(Moshiashvili et al., 2020)
10	DO roots	Used as an antipyretic agent, wound healing agent, lumbago, and against rheumatism.	Turkey	(Moshiashvili et al., 2020)

Table 2. Traditional medicinal uses of *Thymus longicaulis*

Serial Number	Plant parts	Traditional uses	Country of uses	References
1	Whole plant by using the decoction method	Blood pressure regulators (to treat cardiovascular diseases).	Turkey	(Güleç et al., 2023)
2	A perennial herb of TL	Used to cure flu, cold, and cough.	Croatia	(Vladimir-Knežević et al., 2012)
3	An aromatic perennial herb of the plant	Utilized as a remedy to cure colds, nephritis, flu, abdominal pain, and cough.	Italy and Austria	(Galasso et al., 2014)

Table 3. Phytochemistry of *Daphne oleoides subsp. oleoide*

Parts of plant	Preparation types	Phytochemical constituents	Mode of actions	References
Leaves	Methanolic extract	Hesperedin, p-Coumaric acid, Catechin, Gallic acid, Quercetin.	Antibacterial activities	(Safavinia et al., 2021)
Aerial part of DO	Methanolic extract	Daphnetin 8-methyl ether, 4-Ethoxy benzoic acid, 4-Hydroxy benzoic acid	Antimicrobial activity	(Dar et al., 2015)
Aerial part	Ethyl acetate extract	Gnidicin-20-palmitate, 1,2-dehydrodaphnetoxin, Eudesmine, Syringin, oleanolic acid, 3,4-dihydroxy-cinnamic acid docosanolester, Daphnetin, Daphnin, Umbelliferone, etc.	Inhibitory effect on interleukin 1 (IL-1α, IL-1β) and tumour necrosis factor (TNF-α) biosynthesis	(Yeşilada et al., 2001)

Flowering aerial part	Methanolic extract and ethyl acetate subextract	Triumbellin, quercetin-3-*O*-glucoside, and rutarensin.	Wound treating activity	(Süntar et al., 2014)

Table 4. Phytochemistry of *Thymus longicaulis subsp. longicaulis var. Subisophyllus*

Parts of plant	Preparation types	Phytochemical constituents	Mode of actions	References
Aerial part	Ethanolic extract	Hydroxycinnamic acids, Flavonoids, Tannins, Procyanidins, Terpenes, Polyphenols	Anti-AChE and antioxidant activities	(Kindl et al., 2015)
Whole plant	Water, hexane, methanol, and ethyl acetate extracts	γ-terpinene, *p*-cymene, Thymol, Terpinolene, Camphor, Borneol, Myrcene, and α-humulene	Strong antioxidant activity (DPPH, β-carotene/linoleic acid, reducing power and chelating effect tests were done)	(Sarikurkcu et al., 2010)
Leave of TL	The hydro-alcoholic solution was used as extracting solvent	Salvianolic acid K, Rosmarinic acid, Luteolin-*O*-pentoside, Isosalvianolic acid A, and Pinoresinol dihexoside	Antioxidant (DPPH, ABTS, ORAC, and RACI tests were studied for antioxidant properties) and anti-inflammatory effects	(Galasso et al., 2014)
Herb of the TL	Ethyl acetate, phenolic acid, methanol extracts	Rosmarinic acid, Flavonol, Phenolic compounds, Flavonoid,	Antioxidant Activity (radical scavenging on DPPH, β-carotene	(Öztürk, 2015)

			bleaching, and Rancimat methods examinations were done to confirm the effects).	

Table 5. Pharmacological activities of *Daphne oleoides subsp. oleoides*

Activity	Preparation type	Types of study	Testing models	Results	Reference
Potential effect on Pulmonary Fibrosis (PF)	Hydroalcoholic extract of aerial part of (stem+leave) DO	In vivo	Bleomycin (BLM)-induced pulmonary fibrosis in rats.	DO extracts demonstrated a preventive effect on PF by lowering the antioxidant and anti-inflammatory markers.	(Danaei et al., 2023)
Antibacterial property	Hydrodistillation of the aerial part of DO	In vitro	The broth dilution technique was used to examine the antibacterial effect on bacteria	The MIC of DO extract was observed between 25 and 100 µg/mL, which is very significant compared with the standard drug.	(Uysal et al., 2017)

Inhibitory activity	Ethyl acetate extract of aerial part of DO	In vitro	Tests on (IL-1α, IL-1β) and tumor necrosis factor (TNF-α) biosynthesis	The extract demonstrated a prominent inhibitory effect on cytokine and interleukin.	(Yeşilada et al., 2001)
Anti-inflammatory effect	Detoxified DO leave extract (acetic acid)	In vivo	Carrageenan-injected paw edema in Wistar rats test.	The anti-inflammatory action showed significant ($p < 0.001$) on the base dose.	(Rahman & Jahan, 2021)
Anticancer, antioxidant, antimicrobial, and antidiabetic activities	Crude methanolic extracts (CMEs) of roots of DO	In vitro (anticancer, antimicrobial, antioxidant) In vivo (antimicrobial)	Applying the MTT assay method on NIH and HeLa cells. Alloxan-induced rabbits were used for anti-diabetic effect	DO showed distinct inhibition against HeLa cells (IC50: 77.87 μ g/ml) and less cytotoxicity against the NIH cells (IC50: 117.18 μ g/ml). DPPH test (IC50: 52.86 μ g/ml) and DO	(Muddassir et al., 2022)

				exhibited less antidiabetic effect than Met-HCL.	
Wound healing effect	Aerial part of DO methanol extract	In vivo	Linear incision and circular excision wound model in Sprague-Dawley rat.	Quercetin-3-*O*-glucoside was found to be the main active compound for wound curing from the aerial parts of DO.	(Süntar et al., 2014)

Table 6. Pharmacological activities of *Thymus longicaulis*

Activity	Preparation type	Types of study	Testing models	Results	Reference
Antioxidant activity	Methanol, ethyl acetate, and petroleum ether of air-dried herb of TL extract.	In vitro	Antioxidant activities were performed by three examinations: radical scavenging on DPPH, β-carotene bleaching, and Rancimat methods.	A prominent antioxidant effect was observed, illustrating that polyphenols could be this property's accountable constituent.	(Öztürk, 2015)
Antimicrobial effect	Hydrodistillation of the aerial part	In vitro	Microdilution broth assay and standard disc	TL extract showed an antimicrobial	(Vladimir-Knežević

	of the TL extract.		agar diffusion method.	effect against H. influenzae and S. pneumoniae (MIC = 0.78 mg/mL) and less impact against S. aureus (MIC > 25.00 mg/mL)	et al., 2012)
Antioxidant property	Air-dried sample of TL extract of methanol, ethyl acetate, hexane, and water.	In vitro	*β*-carotene/linolei c acid, DPPH, minimizing power, and chelating impact tests were performed to identify antioxidant activity.	TL extracts showed noticeable antioxidant performance in the β-carotene/linol eic acid tests, among others.	(Sarikurkc u et al., 2010)
Anti-inflammatory and antioxidant properties	Hydroalcoholic leaf extract of TL.	In vivo	A COX-2 induction assay was undertaken on human THP-1 monocyte-derived macrophages. ABTS radical cation assays showed 8.91 lg/mL, and DPPH radical	Significant anti-inflammatory and antioxidant effects were exhibited in the Oct 12 extract due to the presence of rosmarinic	(Galasso et al., 2014)

			demonstrated 9.50 lg/mL.	acid and methylapigenin.	

5. MATERIALS and METHODS

5.1. Chemicals used in the experiments

An anti-inflammatory medicine called indomethacin was made of used as a standard medicine in comparison with other groups during the research. Petroleum ether, ethyl acetate, and methanol were used for the preparation of extracts of *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* plant. On the other hand, Chloroform, petroleum ether, and methanol were applied to prepare the crude extracts of *Daphne oleoides* subsp. *oleoides* plants. Carrageenan was administered by the intraplanar (i.p.) route to induce paw edema in Sprague Dawley rats for the acute inflammation examination. Additionally, Ketamine was found to be used for the induction of anesthesia for the rats during the laboratory experiment. Standard rodent feed and tap water were given to the rats. Ethanol was utilized as a disinfectant agent to avoid microbial contamination of the laboratory equipment.

5.2. Machinery implemented in the experiments

The grinder machine was employed to grind the dried plants and prepare the extracts. A Rotary evaporator was used after the maceration technique to gain the crude extracts of the plants from the filtrates. Lyophilizer was applied to obtain the crude extracts from the filtrates of the DO plants. The filter papers were used to filter the extracts. Moreover, curved feeding needles and normal injectable syringes were implemented to administer chemicals to the animals. A plethysmometer was utilized to measure the thickness of the edema formation after inducing carrageenan in rat paws and to observe the decrease of the edema length after administration of test extracts.

5.3. Extraction of plants

5.3.1. Procurement of Plants

The two Turkish traditional medicinal plants such as *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* and *Daphne oleoides* subsp. *oleoides* plants were collected and identified by Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany. Dr. İsmail Şenkardeş is a renowned professor from Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany who has knowledge of the characterization of medicinal plants and has determined the plants as mentioned earlier for pharmacological studies.

5.3.2. Preparation of Extracts

The aerial parts of *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* are dried at room temperature, and the dried plants were crushed into powder. Petroleum ether, ethyl acetate, and

methanol extracts are achieved serially by the maceration process. After finishing the procedure, the liquid part is filtered through filter paper. The solvents of the filtrates are evaporated in a rotary evaporator, and crude extracts are obtained. The acquired crude extracts are stored at +4 °C until the day of the study.

The above-ground parts of *Daphne oleoides* subsp. *oleoides* are dried at room temperature, and the desiccated plants were ground into powder. Petroleum ether, chloroform, and methanol extracts are obtained sequentially by the maceration technique. After completing the maceration process, the liquid part is filtered through filter paper. The solvents of the filtrates are evaporated in a rotary evaporator and lyophilizer to obtain crude extracts. The obtained crude extracts are kept at +4 °C until the day of the study for the research.

5.4. In vivo studies

5.4.1. Designations of animals for the experiments

Thirty male/female Sprague Dawley rats, 12-16 weeks old, weighing 250-350 g, were obtained from Marmara University, Experimental Animal Research Center, and they were properly and carefully maintained in this center. They were fed standard rat chow and tap water as ed libidum. The room conditions of the experimental animals were maintained at 22 ± 2 °C and 45±5% humidity. Animals were kept on a 12-hour dark and 12-hour light cycle. Animals were acclimatized to the environment for 7 days before starting the experiment for the study.

The rats were separated into five groups, and each group contained six rats. The first group (control group) was treated with saline only, the second group (disease group) was treated with carrageenan, the third group (standard group) was applied carrageenan and indomethacin, the fourth group (DO extract treatment group) was injected DO extract orally and the last group (TL extract treatment group) was administered TL extract P.O.

The experimental animals were divided into five groups as follows:

1. **Control group** (Solely Saline treated group): Saline was administered by intraplanar route after 1-hour administration of saline Per-Oral (P.O.).
2. **Disease group** (Carrageenan treated group): Carrageenan was injected by intraplanar route administration of (1% of 0.1 ml (w/v)) after 1-hour administration of saline Per-Oral (P.O.).
3. **Positive control group or standard group** (Carrageenan + Indomethacin (Ind) administration group): Indomethacin (10 mg/kg) was injected by intragastric administration route (i.g.) after 1 hour then injected carrageenan (1% of 0.1 ml (w/v)) by intraplanar route (i.p.) administration.

4. ***Daphne oleoides* subsp. *oleoide* extract treatment group or treatment group**: Carrageenan (1% of 0.1 ml (w/v)) was administered by intraplanar route administration 1 hour after administration of *Daphne oleoides* subsp. *oleoide* extract by per oral (p.o.) route.

5. **Treatment group or *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* extract injected group**: *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* extract was administered per oral (p.o.) route administration and then injected carrageenan (1% of 0.1 ml (w/v)) by intraplanar route administration.

5.4.2. Generalization of rat paw edema

The anti-inflammatory activities of *Daphne oleoides* subsp. *oleoide* and *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* species were carefully investigated by utilizing the carrageenan-induced paw edema method in Sprague Dawley rats. Rats were fasted for 12 hours until the start of the experiment and water was given as ed-libidum. Anti-inflammatory doses of *Daphne oleoides* subsp. *oleoide* and *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* were administered per oral (p.o.) 1 hour before carrageenan administration by the interplanar (i.p) route. In the positive control group, indomethacin was administered by intragastric (i.g.) route 1 hour before carrageenan administration by interplanar route. To induce inflammation, carrageenan (0.1 ml 1% (w/v)) in saline was injected subplanarly way into the right paw of Sprague-Dawley rats. The formed volume and duration of edema development were determined by using a digital device called a plethysmometer at 0, 1, 2, 3, 4, and 5 hours after carrageenan injection for 5 hours. The percentage inhibition of edema was accurately measured and compared to control group animals and was cautiously calculated by applying the following formulation.

$$\% \text{ Inhibition} = [(Vt - Vo)_{\text{control}} - (Vt - Vo)] / [(Vt - Vo)_{\text{control}}] \times 100$$

Where:

Vt is the paw volume at time t,

Vo is the paw volume before carrageenan injection, and

(Vt - Vo) is the edema in the paw after time 't'.

5.5. Statistical analysis

Statistical analysis of the data gained as a result of the measurements was performed by implementing the GraphPad Prism 9 statistical program. The experimental results were given as mean ± standard deviation. Analysis results were provided as mean ± standard deviation,

and intergroup evaluations were made by using one-way and two-way analysis of variance (ANOVA) and Bonferroni post hoc test.

6. RESULTS

In this pharmacological evaluation, the plant extracts of *Daphne oleoides* subsp. *oleoide* (DO) and *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* (TL) were tested for their protective anti-inflammatory activity upon exacerbation of acute inflammation in carrageenan-mediated paw edema caused by carrageenan in Sprague Dawley rats. The findings are drawn up with the graphs below in Figure 11. In the experiments, paw volume (ml) and paw thickness (mm) were primarily measured at 0, 1, 2, 3, and 4 hours to assess the acute inflammation effects in rats.

In Figure 11 (a), paw volume is mentioned with the other groups of the experiments. In this figure, paw volumes were the same for every group at 0 hours, but the control group demonstrated the progressive enhancement in paw volume compared with other groups, peaking at 3 hours with the volume of approximately 2.6 ml after carrageenan injection and declining slightly at 4 hours. The INDO, DO, and TL groups showed a lower increment of paw volume compared with the control groups at 3 and 4 hours. The reference drug indomethacin (NSAID) group exhibited a critical ($p< 0.01$) demotion in paw volume beginning at 3 hours. The DO extract reduced the swelling notably ($p< 0.05$) but slightly less than the INDO and TL groups. On the other part, the group of TL performed a promising effect on the reduction of paw volume significantly ($p<0.01$) by more than 10 % in every hour starting from 2 hours. In this paw volume test, the TL extract exhibited slightly greater anti-inflammatory properties on acute inflammation than the standard drug indomethacin, which is quite noticeable for therapeutic uses. At the 4-hour point, the TL and DO extract reduce the paw volume to approximately 1.4 ml and 1.7 ml, respectively.

In Figure 11 (b), paw thickness is measured by using a device called a plethysmometer after carrageenan administration during the experiment at 0, 1, 2, 3, and 4 hours to investigate the protective effect on acute inflammation in rats. In this experiment, the control group illustrated a steady enhancement of the hourly thickness, and the maximum peak level was at 4 hours with nearly 8.2 mm thickness, contrasting with the other groups. The three groups, INDO, TL, and DO, reveal a gradual decline in thickness at 2 hours, contrary to the control. The standard medicine, indomethacin group, reveals a noticeable ($p< 0.001$) reduction in thickness than the remaining test group. The test group of DO displays an insignificant ($p< 0.05$) lower effect than the TL and the standard groups. The TL group established a significant ($p< 0.001$) lessening of the thickness to around 6 mm, nearly the same as the standard medicine, indomethacin. Both TL and DO plant extracts begin showing protective effects on acute inflammation by lowering paw volume and paw thickness, almost after 2 hours of administration. In both paw volume and paw thickness evaluations, the plant extract of TL showed a dominant effect of potential

protection on acute inflammation in rats. Both plant extracts illustrated the same effect on decreasing paw thickness at 2 hours. At the 4-hour mark, the TL and DO extracts decreased the paw thickness to nearly 5.9 mm and 6.1 mm, respectively.

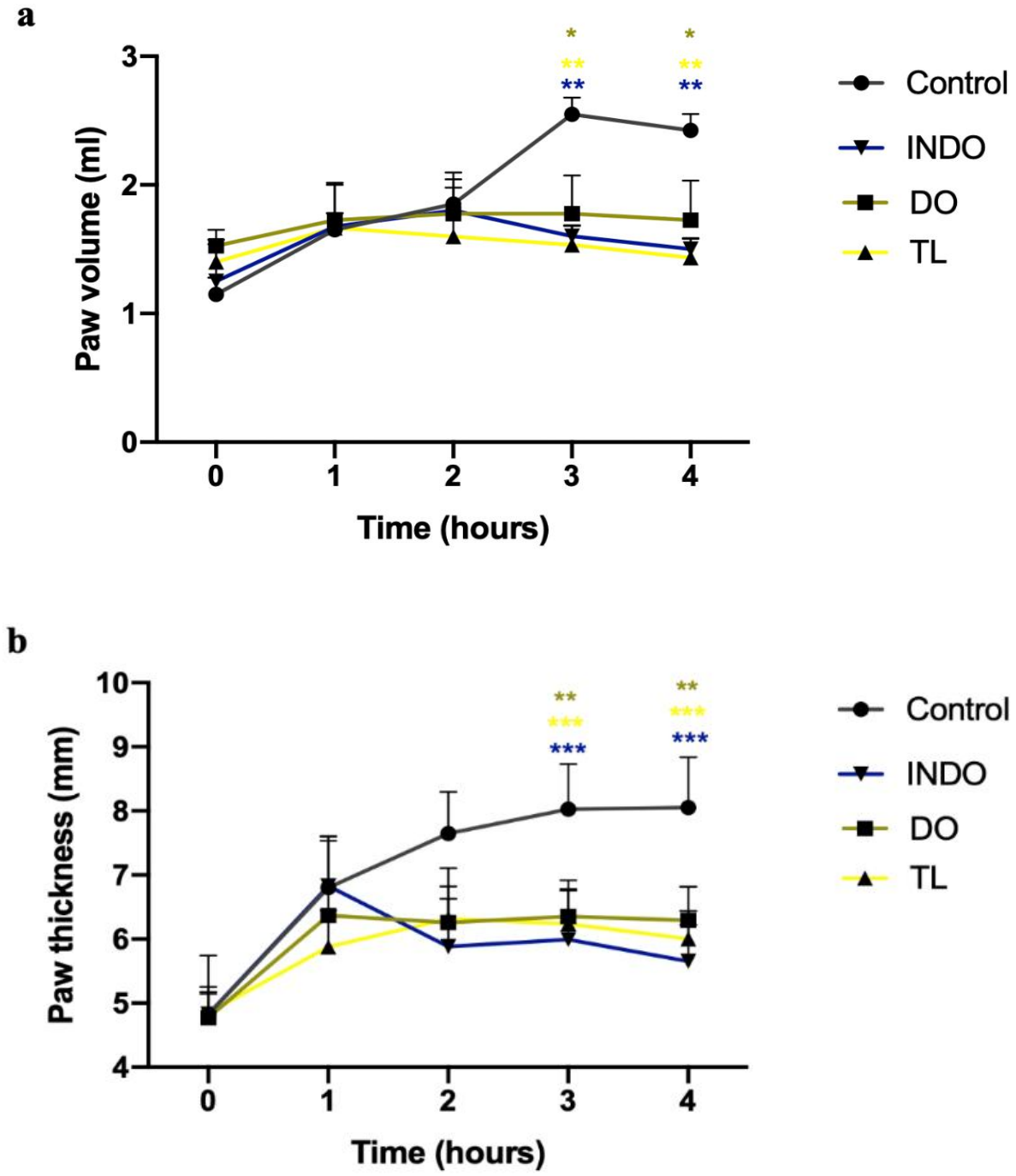


Figure 11. (a) Paw volume (ml) of rats; (b) Paw thickness (mm) of rats. *p <0.05, **p <0.01, ***p <0.001 in comparison to the control group; $^{\#}$p <0.05, $^{\#\#}$p <0.01, $^{\#\#\#}$p <0.001 in comparison to the INDO group.

7. DISCUSSION

In this in vivo pharmacological study, the plant extracts of *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* (TL) and *Daphne oleoides* subsp. *oleoide* (DO) showed a protective effect on acute inflammation by reducing the paw volume and thickness in carrageenan-injected Sprague-Dawley rats. The TL extract was more effective than the DO plant extract in controlling acute inflammation. Moreover, TL extract demonstrated a significant defensive effect by lowering the volume and thickness of paw edema, which is nearly the same as the indomethacin effects. This shows that TL possesses a securing and promising effect on treating acute inflammation. A preliminary study has previously established that the aerial part of the methanolic extract of the *Daphne oleoides* plant (five sub-extracts such as dichloromethane (CH_2Cl_2), *n*-hexane, ethyl acetate, *n*-butanol, and the remaining water) possesses antimicrobial, anti-oxidant agents and anti-inflammatory activity in an in vitro assay model and additionally claimed that the presence of flavonoid and phenolic content may be primarily responsible for these effects (Balkan et al., 2017). Another in vitro investigation exhibited that the above-ground part or root of *Daphne oleoides* is applied as a folk medicine to treat diverse types of disease, such as fever, rheumatic pain, and lumbago. This study also proved that ethyl acetate, hexane, and chloroform extract DO revealed inhibitory activity on some inflammatory mediators such as IL-1β, IL-1α, TNF-α, and IL-1 biosynthesis (Yeşilada et al., 2001). A prominent gastrointestinal study has shown that the methanolic crude extract of DO plant has spasmolytic and spasmogenic properties, which demonstrate that this plant is useful for the management of gastric motility problems. This study validated that the gut stimulation and inhibitory properties of DO were gained through Ca^{++} antagonistic and cholinergic pathways via triggering muscarinic receptors (Khan et al., 2011). An animal model study revealed that the wound healing property of DO extract was gained due to the presence of luteolin-7-*O*-glucoside by applying circular excision and linear incision methods. Besides that, it was also illustrated that this plant extract showed anti-hyaluronidase, antioxidant, anti-collagenase, and anti-inflammatory properties (Süntar et al., 2012).

On the other way, the plant *Thymus longicaulis* (TL) is useful as a folkloric pharmaceutical to treat numerous types of problems, including cold, abdominal pain, cough, flu, and nephritis. In the TL extract experiments, the following pharmacological activities were found particularly anti-inflammatory, antioxidant, and antimicrobial. Antioxidant activity was evaluated due to the presence of profound essential oils in the *β*-carotene/linoleic acid among all the tests done (Galasso et al., 2014; Sarikurkcu et al., 2010; Vladimir-Knežević et al., 2012). Another study also exhibited that the aqueous extract of the TL plant could be utilized as a natural antioxidant

agent, which is very effective in maintaining the physiological stress of cells and organs and protects from developing serious diseases (Zuleyha, 2020). Another important neuropharmacological study found that the ethanolic extract of TL is quite effective in managing oxidative stress and neurological disorders like Alzheimer's disease by suppressing the acetylcholinesterase enzyme because of terpenes and polyphenols present in the species (Kindl et al., 2015).

By analyzing the data and information acquired from the experiments and researching earlier study sources, it can be said that *Daphne oleoides* and *Thymus longicaulis* possess anti-inflammatory activity. The botanical extracts displayed considerable anti-inflammatory qualities by downsizing the amount of edema and minimizing the extent of paw thickness. Moreover, the TL extract showed a notable effect by reducing the edema volume by approximately 10% every hour, underscoring its potential effect as an anti-inflammation agent. This observation illustrates the alignment with the previous research that underlined the inflammatory activities of plant-derived phytochemical compounds that frequently play through the regularization of the cytokine composition, cyclooxygenase enzyme inhibition (COX), and reduction of oxidative stress. It is a complex pathophysiological pathway, and plant-based treatment protocols frequently provide multiple approaches due to numerous phytochemical compounds, including flavonoids, polyphenols, terpenes, alkaloids, tannins, saponins, organic acids, etc. Earlier findings have suggested that these biologically active components are primarily concerned with downregulating the inflammatory cascade by focusing on inflammatory intermediaries such as nitric oxide, IL-1β, prostaglandins, IL-1α, thromboxane A2, TNF-α, and IL-1. The DO and TL plant extracts exhibited a potential protective effect on acute inflammation as promising therapeutic agents to treat inflammatory disorders. Additionally, most of the previous research was conducted in vitro, which is a gap in fully understanding the pharmacological properties of these species. For this reason, this work is emphasized on an animal model experiment to investigate the beneficial effect on the acute inflammation in Sprague Dawley rats administered carrageenan. Both DO and TL plant extracts exhibited prominent effects on protecting against acute inflammation, but the TL plant showed a superior potential therapeutic effect on treating acute inflammation than the DO plant extract. This indicated that TL plant extract was more powerful than DO herbal extract in modulating acute inflammation in carrageenan-exposed paw edema in Sprague Dawley rats.

7.1. CONCLUSION

Medicinal plants are assumed to be the primary source of potential crude drugs for treating various diseases worldwide. Most drugs are derived from plants and are very effective for emerging untreatable diseases. In our study, we have studied two medicinal plants called *Daphne oleoides* subsp. *oleoide* (DO) and *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* (TL). Both plants have been utilizing for many purposes, including traditional, therapeutic, pharmaceutical, and food additives. The DO species are traditionally used to treat fever, rheumatism, wounds, malaria, lumbago, laxative agents, ulcers, and skin problems. On the other hand, the plant TL is also used as folk medicine for treating cardiovascular disorders, colds, coughs, abdominal pain, nephritis, and flu. Numerous pharmacological studies have exhibited that both species have anti-inflammation properties, antioxidant, antitumor, anticancer, antifungal, antipyretic, antihypertensive, antidiabetic, etc. Several phytochemical studies have discovered many bioactive compounds that could be accountable for specific diseases and others. In our experiment, it was found that both DO and TL species significantly affected treating acute inflammation on Sprague Dawley rat paws induced by carrageenan. In contrast, TL herbal extract was reported to be superior to DO extract in protecting against acute inflammation. Therefore, by considering the outcomes of this study, it can be said that the TL and DO plants could be useful as a therapeutic agent to treat acute inflammation. In conclusion, further animal model pharmacological studies are required due to limited research on animals to completely comprehend these two medicinal plants. However, more studies should be conducted to investigate and identify the accountable phytochemical compound for the properties of acute inflammation treatment and to understand the mechanism of action, long-term safety, efficacy, toxicity parameters, and porosity studies before presenting them for the trials in humans.

8. REFERENCES

Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., & Martins, C. H. G. (2023). Back to Nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*, *12*(17), 3077. https://doi.org/10.3390/plants12173077

Abdulkhaleq, L., Assi, M., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y., & Hezmee, M. (2018). The crucial roles of inflammatory mediators in inflammation: A review. Veterinary World, 11 (5), 627–635. In. https://doi.org/10.14202/vetworld.2018.627-635

Abduraimov, O., Li, W., Shomurodov, H., & Feng, Y. (2023). The Main Medicinal Plants in Arid Regions of Uzbekistan and Their Traditional Use in Folk Medicine. Plants, 12, Article No. 2950. In. https://doi.org/10.3390/plants12162950

Ahmed, A. U. (2011). An overview of inflammation: mechanism and consequences. *Frontiers in Biology*, *6*(4), 274-281. https://doi.org/10.1007/s11515-011-1123-9

Anwar, F., Mahrye, Khan, R., Qadir, R., Saadi, S., Gruczynska-Sekowska, E., Saari, N., & Hossain Brishti, F. (2024). Exploring the Biochemical and Nutra-Pharmaceutical Prospects of Some Thymus Species–A Review. *Chemistry & biodiversity*, *21*(7), e202400500. https://doi.org/10.1002/cbdv.202400500

Balandrin, M. F., Kinghorn, A. D., & Farnsworth, N. R. (1993). Plant-derived natural products in drug discovery and development: an overview. https://doi.org/10.1021/bk-1993-0534.ch001

Balkan, İ. A., Taşkın, T., Doğan, H. T., Deniz, İ., Akaydın, G., & Yesilada, E. (2017). A comparative investigation on the in vitro anti-inflammatory, antioxidant and antimicrobial potentials of subextracts from the aerial parts of Daphne oleoides Schreb. subsp. oleoides. *Industrial crops and products*, *95*, 695-703. http://dx.doi.org/10.1016/j.indcrop.2016.11.038

Banerjee, A., Dwivedi, M. D., Kasana, S., Singh, P., Kumar, V., Maity, D., & Pandey, A. K. (2022). Phylogenetic relationships in Indian Daphne (Thymelaeaceae) based on nuclear ITS and cpDNAdata. *Biologia*, *77*(11), 3071-3086. https://doi.org/10.1007/s11756-022-01125-4

Bartolucci, F., & Domina, G. (2014). Typification and taxonomic characterization of Thymus longicaulis C. Presl (Lamiaceae). Annales Botanici Fennici, https://doi.org/10.5735/085.051.0107

Baytop, T. (1999). Therapy with medicinal plants in Turkey (past and present). *Publication of the istanbul University*, *312*, 2-3.

Bidian, C., Filip, G. A., David, L., Moldovan, B., Baldea, I., Olteanu, D., Filip, M., Bolfa, P., Potara, M., & Toader, A. M. (2022). Viburnum opulus fruit extract-capped gold nanoparticles attenuated oxidative stress and acute inflammation in carrageenan-induced paw edema model. *Green Chemistry Letters and Reviews*, *15*(2), 320-336. http://dx.doi.org/10.1080/17518253.2022.2061872

Calder, P. C., Ahluwalia, N., Albers, R., Bosco, N., Bourdet-Sicard, R., Haller, D., Holgate, S. T., Jönsson, L. S., Latulippe, M. E., & Marcos, A. (2013). A consideration of biomarkers

to be used for evaluation of inflammation in human nutritional studies. *British Journal of Nutrition*, *109*(S1), S1-S34. https://doi.org/10.1017/s0007114512005119

Campolo, O., Zappalà, L., Malacrinò, A., Laudani, F., & Palmeri, V. (2016). Bees visiting flowers of Thymus longicaulis (Lamiaceae). *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *150*(6), 1182-1188. http://dx.doi.org/10.1080/11263504.2015.1009190

Chen, Z., Ding, W., Yang, X., Lu, T., & Liu, Y. (2024). Isoliquiritigenin, a potential therapeutic agent for treatment of inflammation-associated diseases. *Journal of ethnopharmacology*, *318*, 117059. https://doi.org/10.1016/j.jep.2023.117059

Danaei, N., Sadeghi, H., Asfaram, A., Rostamzadeh, D., Sadeghi, H., & Rahimi, N. (2023). Betulin-rich hydroalcoholic extract of Daphne oleoides attenuates bleomycin-induced pulmonary fibrosis in rat. *Heliyon*, *9*(8). https://doi.org/10.1016/j.heliyon.2023.e19236

Dar, M. Y., Ara, T., & Akbar, S. (2015). Isolation of daphnetin 8-methyl ether from Daphne oleoides and its anti-bacterial activity. *J. Phytopharm*, *4*(4), 224-226. https://phytopharmajournal.com/articles/details/521

Dinarello, C. A. (2010). Anti-inflammatory agents: present and future. *Cell*, *140*(6), 935-950. https://doi.org/10.1016/j.cell.2010.02.043

Du, W. J., Yang, X. L., Song, Z. J., Wang, J. Y., Zhang, W. J., He, X., Zhang, R. Q., Zhang, C. F., Li, F., & Yu, C. H. (2016). Antitumor activity of total flavonoids from Daphne genkwa in colorectal cancer. *Phytotherapy Research*, *30*(2), 323-330. https://doi.org/10.1002/ptr.5540

Elkıran, Ö. (2022). Chemical Composition of the Essential Oil of Thymus longicaulis C. Presl. subsp. longicaulis. *International Journal of Secondary Metabolite*, *9*(3), 248-257. http://dx.doi.org/10.21448/ijsm.1086904

Eroglu Ozkan, E., Ersoy, E., Yesil Canturk, Y., Mataraci Kara, E., Cinar, E., Sahin, H., Karahan, S., Karaca Sancaktepe, K., Yilmaz, M. A., & Boga, M. (2022). The therapeutic potential of Ethnomedicinally important Anatolian Thyme species: a Phytochemical and Biological Assessment. *Frontiers in pharmacology*, *13*, 923063. http://dx.doi.org/10.3389/fphar.2022.923063

Ferreira, T., Faustino-Rocha, A. I., Gaspar, V. M., Medeiros, R., Mano, J. F., & Oliveira, P. A. (2024). Contribution of non-steroidal anti-inflammatory drugs to breast cancer treatment: In vitro and in vivo studies. *Veterinary World*, *17*(5), 1052. https://doi.org/10.14202/vetworld.2024.1052-1072

Fujita, T., Sezik, E., Tabata, M., Yeşilada, E., Honda, G., Takeda, Y., Tanaka, T., & Takaishi, Y. (1995). Traditional medicine in Turkey VII. Folk medicine in middle and west Black Sea regions. *Economic botany*, 406-422. http://dx.doi.org/10.1007/BF02863092

Galasso, S., Pacifico, S., Kretschmer, N., Pan, S.-P., Marciano, S., Piccolella, S., Monaco, P., & Bauer, R. (2014). Influence of seasonal variation on Thymus longicaulis C. Presl chemical composition and its antioxidant and anti-inflammatory properties. *Phytochemistry*, *107*, 80-90. https://doi.org/10.1016/j.phytochem.2014.08.015

Ghasemi Pirbalouti, A., Emami Bistghani, Z., & Malekpoor, F. (2015). An overview on genus Thymus. *Journal of Medicinal Herbs*, *6*(2), 93-100.

Güleç, M., Erarslan, Z. B., & Kültür, Ş. (2023). The Medicinal Plants Traditionally Used Against Cardiovascular Diseases in Türkiye. *International Journal of Traditional and Complementary Medicine Research*, *4*(2), 81-96. http://dx.doi.org/10.53811/ijtcmr.1232190

Hashim, S., & Gamil, M. (1988). Plants and herbs between the Iraqi folk medicine and scientific research. *Baghdad, Dar revolution of Press and Publication*.

Hossain, M. K., Prodhan, M. A., Even, A. I. H., Morshed, H., & Hossain, M. M. (2012). Anti-inflammatory and antidiabetic activity of ethanolic extracts of Sterculia villosa barks on Albino Wistar rats. *Journal of Applied Pharmaceutical Science*, *2*(8), 96-100. http://dx.doi.org/10.7324/JAPS.2012.2815

Hou, Z.-l., Yao, G.-d., & Song, S.-j. (2021). Daphnane-type diterpenes from genus Daphne and their anti-tumor activity. *Chinese Herbal Medicines*, *13*(2), 145-156. https://doi.org/10.1016/j.chmed.2020.09.006

Jamzad, Z. (2010). Thymus and Satureja spp of Iran. *Research Institute of Forests and Rangelands Press, Tehran*.

Karahan, F. (2023). Evaluation of trace element and heavy metal levels of some ethnobotanically important medicinal plants used as remedies in Southern Turkey in terms of human health risk. *Biological trace element research*, *201*(1), 493-513. https://doi.org/10.1007/s12011-022-03299-z

Khan, A.-u., Ali, F., Khan, D., & Gilani, A.-H. (2011). Gut modulatory effects of Daphne oleoides are mediated through cholinergic and Ca++ antagonist mechanisms. *Pharmaceutical Biology*, *49*(8), 821-825. https://doi.org/10.3109/13880209.2010.550056

Khodayari, H., Faramarzi, A., & Jalilian, N. (2017). The Morphological, Micromorphological and Palynological Study of the Genus Daphne L.(Thymelaeacea) in Iran. *Taxonomy and Biosystematics*, *9*(33), 47-64. https://doi.org/10.22108/tbj.2018.107967.1046

Khumalo, G. P., Van Wyk, B. E., Feng, Y., & Cock, I. E. (2022). A review of the traditional use of southern African medicinal plants for the treatment of inflammation and inflammatory pain. *Journal of ethnopharmacology*, *283*, 114436. https://doi.org/10.1016/j.jep.2021.114436

Kindl, M., Blažeković, B., Bucar, F., & Vladimir-Knežević, S. (2015). Antioxidant and anticholinesterase potential of six thymus species. *Evidence-Based Complementary and Alternative Medicine*, *2015*(1), 403950. https://doi.org/10.1155/2015/403950

Laurindo, L. F., de Maio, M. C., Minniti, G., de Góes Corrêa, N., Barbalho, S. M., Quesada, K., Guiguer, E. L., Sloan, K. P., Detregiachi, C. R., & Araújo, A. C. (2023). Effects of medicinal plants and phytochemicals in Nrf2 pathways during inflammatory bowel diseases and related colorectal cancer: A comprehensive review. *Metabolites*, *13*(2), 243. https://doi.org/10.3390/metabo13020243

Lawrence, T. (2009). The nuclear factor NF-κB pathway in inflammation. *Cold Spring Harbor perspectives in biology*, *1*(6), a001651.

https://doi.org/10.1101/cshperspect.a001651
Li, X., He, T., Wang, X., Shen, M., Yan, X., Fan, S., Wang, L., Wang, X., Xu, X., & Sui, H. (2019). Traditional uses, chemical constituents and biological activities of plants from the genus Thymus. *Chemistry & biodiversity*, *16*(9), e1900254. https://doi.org/10.1002/cbdv.201900254
Libby, P. (2007). Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutrition reviews*, *65*(suppl_3), S140-S146. https://doi.org/10.1111/j.1753-4887.2007.tb00352.x
Liu, Y.-Z., Wang, Y.-X., & Jiang, C.-L. (2017). Inflammation: the common pathway of stress-related diseases. *Frontiers in human neuroscience*, *11*, 273283. https://doi.org/10.3389/fnhum.2017.00316
Lucas, S. (2016). The pharmacology of indomethacin. *Headache: The Journal of Head and Face Pain*, *56*(2), 436-446. https://doi.org/10.1111/head.12769
Madkhali, J. Y., Hussein, R. H., & Alnahdi, H. S. (2023). Therapeutic effect of bromelain and papain on intestinal injury induced by indomethacin in male rats. *International Journal of Health Sciences*, *17*(5), 23.
McGEER, P. L., & McGEER, E. G. (2004). Inflammation and the degenerative diseases of aging. *Annals of the New York Academy of Sciences*, *1035*(1), 104-116. https://doi.org/10.1196/annals.1332.007
Miroslav, M., Sovrlić, M., & Manojlović, N. (2016). Plants from the Genus Daphne: a review of its traditional uses, phytochemistry, biological and pharmacological activity. *Ser J Exp Clin Res*, *17*, 1-1. http://dx.doi.org/10.1515/sjecr-2016-0024
Misra, H., Mehta, B. K., & Jain, D. C. (2008). Optimization of Extraction Conditions and HPTLC-UV Method for Determination of Quinine in Different Extracts of Cinchona species Bark. *Records of Natural Products*, *2*(4).
Moshiashvili, G., Tabatadze, N., & Mshvildadze, V. (2020). The genus Daphne: A review of its traditional uses, phytochemistry and pharmacology. *Fitoterapia*, *143*, 104540. https://doi.org/10.1016/j.fitote.2020.104540
Muddassir, M., Batool, A., Miana, G. A., & Zafar, S. (2022). Comparative studies of anticancer, antimicrobial, antidiabetic, antioxidant activities of Daphne oleoides and Berberis baluchistanica extracts native to Pakistan. *Pakistan Journal of Pharmaceutical Sciences*, *35*(2), 649-656. http://dx.doi.org/10.36721/PJPS.2022.35.2.SP.649-656.1
Muzamil, A., Tahir, H. M., Ali, S., Liaqat, I., Ali, A., & Summer, M. (2021). Inflammatory process and role of cytokines in inflammation: An overview. *Punjab Univ. J. Zool*, *36*(10.17582). https://dx.doi.org/10.17582/journal.pujz/2021.36.2.237.252
Nalamachu, S., & Wortmann, R. (2014). Role of indomethacin in acute pain and inflammation management: a review of the literature. *Postgraduate medicine*, *126*(4), 92-97. https://doi.org/10.3810/pgm.2014.07.2787
Nie, Y.-W., Li, Y., Luo, L., Zhang, C.-Y., Fan, W., Gu, W.-Y., Shi, K.-R., Zhai, X.-X., & Zhu, J.-Y. (2021). Phytochemistry and pharmacological activities of the diterpenoids from the genus Daphne. *Molecules*, *26*(21), 6598.

https://doi.org/10.3390/molecules26216598

Ong, C., Lirk, P., Tan, C., & Seymour, R. (2007). An evidence-based update on nonsteroidal anti-inflammatory drugs. *Clinical medicine & research*, *5*(1), 19-34. https://doi.org/10.3121/cmr.2007.698

Otake, H., Ogata, F., Nakazawa, Y., Misra, M., Tsubaki, M., Kawasaki, N., & Nagai, N. (2025). Pharmacological Behavior of Propylene Glycol/Polyvinyl Alcohol Hydrogel Incorporating Indomethacin Nanocrystals in the Skin. *Gels*, *11*(4), 251. https://doi.org/10.3390/gels11040251

Ou, Z., Zhao, J., Zhu, L., Huang, L., Ma, Y., Ma, C., Luo, C., Zhu, Z., Yuan, Z., & Wu, J. (2019). Anti-inflammatory effect and potential mechanism of betulinic acid on λ-carrageenan-induced paw edema in mice. *Biomedicine & Pharmacotherapy*, *118*, 109347. https://doi.org/10.1016/j.biopha.2019.109347

Öztürk, N. (2015). Phenolic composition and antioxidant activity of the different extracts from Thymus longicaulis C Presl. subsp. longicaulis var. longicaulis and T. longicaulis C. Presl. subsp. longicaulis var. subisophyllus growing in Turkey. *Pakistan Journal of Pharmaceutical Sciences*, *28*(2).

Punchard, N. A., Whelan, C. J., & Adcock, I. (2004). The journal of inflammation. In (Vol. 1, pp. 1-4): Springer. https://doi.org/10.1186/1476-9255-1-1

Rahman, S., & Jahan, N. (2021). Anti-inflammatory activity of crude and detoxified leaves of Mazaryun (Daphne oleoides Schreb.) on carrageenan-induced paw edema in wistar rats. https://doi.org/10.1016/j.jaim.2021.04.016

Rao, P., & Knaus, E. E. (2008). Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *Journal of pharmacy & pharmaceutical sciences*, *11*(2), 81s-110s. https://doi.org/10.18433/j3t886

Riaz, M., Saleem, A., Siddique, S., Khan, B. A., Nur-e-Alam, M., Shahzad-ul-Hussan, S., Miana, G. A., & Khan, M. Q. (2016). Phytochemistry of Daphne oleoides. *Natural product research*, *30*(8), 880-897. https://doi.org/10.1080/14786419.2015.1092146

Rousdy, D. W., Wardoyo, E. R. P., & Ifadatin, S. (2022). Anti-inflammatory Activity of Bajakah Stem (Spatholobus littoralis Hassk.) Ethanolic Extract in Carrageenan-Induced Paw Edema Mice. *Jurnal Biodjati*, *7*(1), 66-74. http://dx.doi.org/10.15575/biodjati.v7i1.14126

Safavinia, L., Akhgar, M. R., Tahamipour, B., & Ahmadi, S. A. (2021). Green synthesis of highly dispersed zinc oxide nanoparticles supported on silica gel matrix by Daphne oleoides extract and their antibacterial activity. *Iranian Journal of Biotechnology*, *19*(1), e2598. https://doi.org/10.30498/ijb.2021.2598

Salehi, B., Abu-Darwish, M. S., Tarawneh, A. H., Cabral, C., Gadetskaya, A. V., Salgueiro, L., Hosseinabadi, T., Rajabi, S., Chanda, W., & Sharifi-Rad, M. (2019). Thymus spp. plants-Food applications and phytopharmacy properties. *Trends in Food Science & Technology*, *85*, 287-306. http://dx.doi.org/10.1016/j.tifs.2019.01.020

Sanda, M. A., Zengin, G., Aktümsek, A., & Cakmak, Y. S. (2015). Evaluation of antioxidant potential of two Daphne species (D. gnidioides and D. pontica) from Turkey. http://dx.doi.org/10.9755/ejfa.2015.04.030

Sarikurkcu, C., Ozer, M. S., Eskici, M., Tepe, B., Can, Ş., & Mete, E. (2010). Essential oil composition and antioxidant activity of Thymus longicaulis C. Presl subsp. longicaulis var. longicaulis. *Food and Chemical Toxicology*, *48*(7), 1801-1805. https://doi.org/10.1016/j.fct.2010.04.009

Scott, A., Khan, K., Cook, J., & Duronio, V. (2004). What is "inflammation"? Are we ready to move beyond Celsus? *British journal of sports medicine*, *38*(3), 248-249. https://doi.org/10.1136/bjsm.2003.011221

Serhan, C. N., Dalli, J., Colas, R. A., Winkler, J. W., & Chiang, N. (2015). Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1851*(4), 397-413. https://doi.org/10.1016/j.bbalip.2014.08.006

Sitoe, E., & Van Wyk, B.-E. (2024). An inventory and analysis of the medicinal plants of Mozambique. *Journal of ethnopharmacology*, *319*, 117137. https://doi.org/10.1016/j.jep.2023.117137

Sobhani, K., Li, J., & Cortes, M. (2023). Nonsteroidal anti-inflammatory drugs (NSAIDs). In *First Aid Perioperative Ultrasound: Acute Pain Manual for Surgical Procedures* (pp. 127-138). Springer. https://doi.org/10.1007/978-3-031-21291-8_8

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, *10*(5), 210-229. https://doi.org/10.4314/ajtcam.v10i5.2

Sohail, R., Mathew, M., Patel, K. K., Reddy, S. A., Haider, Z., Naria, M., Habib, A., Abdin, Z. U., Chaudhry, W. R., & Akbar, A. (2023). Effects of non-steroidal anti-inflammatory drugs (NSAIDs) and gastroprotective NSAIDs on the gastrointestinal tract: a narrative review. *Cureus*, *15*(4). https://doi.org/10.7759/cureus.37080

Sophia, D., Gopalakrishnan, V., Ram Kumar, C., & Vijayalakshmi, B. (2023). An Insight into Traditional and Integrative Medicine. In *Translating Healthcare Through Intelligent Computational Methods* (pp. 37-48). Springer. https://doi.org/10.1007/978-3-031-27700-9_3

Stahl-Biskup, E., & Venskutonis, R. P. (2012). Thyme. In *Handbook of herbs and spices* (pp. 499-525). Elsevier. https://doi.org/10.1533/9780857095671.499

Stankov, S. V. (2012). Definition of inflammation, causes of inflammation and possible anti-inflammatory strategies. *Open Inflamm J*, *5*(1), 1-9. http://dx.doi.org/10.2174/1875041901205010001

Stone, W. L., Basit, H., & Burns, B. (2018). Pathology, inflammation.

Süntar, I. (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews*, *19*(5), 1199-1209. https://link.springer.com/article/10.1007/s11101-019-09629-9

Süntar, I., Akkol, E. K., Keles, H., Yesilada, E., Sarker, S., & Baykal, T. (2014). Daphne oleoides Schreber ssp. oleoides Exhibits Potent Wound Healing Effect: Isolation of the Active Components and Elucidation of the Activity Mechanism. *Records of Natural Products*, *8*(2).

Süntar, I., Akkol, E. K., Keles, H., Yesilada, E., Sarker, S. D., Arroo, R., & Baykal, T. (2012). Efficacy of Daphne oleoides subsp. kurdica used for wound healing: identification of active compounds through bioassay guided isolation technique. *Journal of ethnopharmacology*, *141*(3), 1058-1070. https://doi.org/10.1016/j.jep.2012.04.001

Taylor, J., Rabe, T., McGaw, L., Jäger, A., & Van Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. *Plant growth regulation*, *34*, 23-37. https://doi.org/10.1023/A:1013310809275

Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology*, *7*(1), 65-74. https://doi.org/10.2174/157015909787602823

Uysal, A., Zengin, G., Aktumsek, A., Rigano, D., Senatore, F., & Sanda, M. A. (2017). Daphne oleoides: An alternative source of important sesquiterpenes. *International Journal of food properties*, *20*(3), 549-559. http://dx.doi.org/10.1080/10942912.2016.1168836

Van Vuuren, S., & Muhlarhi, T. (2017). Do South African medicinal plants used traditionally to treat infections respond differently to resistant microbial strains? *South African journal of botany*, *112*, 186-192. https://doi.org/10.1016/j.sajb.2017.05.027

Vane, J. R., & Botting, R. M. (1998). Anti-inflammatory drugs and their mechanism of action. *Inflammation Research*, *47*, 78-87. https://doi.org/10.1007/s000110050284

Venditti, A., Sanna, C., Lorenzetti, L. M., Ballero, M., & Bianco, A. (2017). New coumarinyl ethers in Daphne oleoides Schreb. Collected from Sardinia island. *Chemistry & biodiversity*, *14*(6), e1700072. https://doi.org/10.1002/cbdv.201700072

Venditti, A., Serrilli, A. M., Di Cecco, M., Ciaschetti, G., & Bianco, A. (2019). Coumarins and other components of Daphne oleoides Schreb. subsp. oleoides from Majella National Park. *Biochemical Systematics and Ecology*, *83*, 39-46. https://doi.org/10.1016/j.bse.2018.12.013

Vladimir-Knežević, S., Kosalec, I., Babac, M., Petrović, M., Ralić, J., Matica, B., & Blažeković, B. (2012). Antimicrobial activity of Thymus longicaulis C. Presl essential oil against respiratory pathogens. *Central European Journal of Biology*, *7*, 1109-1115. https://doi.org/10.2478/s11535-012-0088-2

Wongrakpanich, S., Wongrakpanich, A., Melhado, K., & Rangaswami, J. (2017). A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging Dis. 9: 143-150. In. https://doi.org/10.14336/ad.2017.0306

Yeşilada, E., Honda, G., Sezik, E., Tabata, M., Fujita, T., Tanaka, T., Takeda, Y., & Takaishi, Y. (1995). Traditional medicine in Turkey. V. Folk medicine in the inner Taurus Mountains. *Journal of ethnopharmacology*, *46*(3), 133-152. https://doi.org/10.1016/0378-8741(95)01241-5

Yeşilada, E., Taninaka, H., Takaishi, Y., Honda, G., Sezik, E., Momota, H., Ohmoto, Y., & Taki, T. (2001). In vitro inhibitory effects of Daphne oleoides ssp. oleoides on inflammatory cytokines and activity-guided isolation of active constituents. *Cytokine*, *13*(6), 359-364. https://doi.org/10.1006/cyto.2001.0838

Yousseu Nana, W., Billong Mimb, J. R., Atsamo, A. D., Tsafack, E. G., Djuichou Nguemnang, S. F., Fagni Njoya, Z. L., Matah Marthe, V. M., Madjo Kouam, Y. K., Mbiantcha, M., & Ateufack, G. (2023). In Vitro and In Vivo Anti-Inflammatory Properties of the Hydroethanolic Extract of the Roots of Vernonia guineensis (Asteraceae). *International Journal of Inflammation*, *2023*. https://doi.org/10.1155/2023/7915367

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*(5), 559. https://doi.org/10.3390/molecules21050559

Zafar, F., Asif, H. M., Shaheen, G., Ghauri, A. O., Rajpoot, S. R., Tasleem, M. W., Shamim, T., Hadi, F., Noor, R., & Ali, T. (2023). A comprehensive review on medicinal plants possessing antioxidant potential. *Clinical and Experimental Pharmacology and Physiology*, *50*(3), 205-217. https://doi.org/10.1111/1440-1681.13743

Zhan, Z.-J., Fan, C.-Q., Ding, J., & Yue, J.-M. (2005). Novel diterpenoids with potent inhibitory activity against endothelium cell HMEC and cytotoxic activities from a well-known TCM plant Daphne genkwa. *Bioorganic & medicinal chemistry*, *13*(3), 645-655. https://doi.org/10.1016/j.bmc.2004.10.054

Zhang, C.-Y., Luo, L., Xia, J., Song, Y.-N., Zhang, L.-J., Zhang, M., Rahman, K., Ye, Y., Zhang, H., & Zhu, J.-Y. (2018). Sesquiterpenes and lignans from the flower buds of Daphne genkwa and their nitric oxide inhibitory activities. *Natural product research*, *32*(24), 2893-2899. https://doi.org/10.1080/14786419.2017.1389937

Zuleyha, O. (2020). Antioxidant activities of aqueous extracts from two Thymus species growing in Turkiye. *GSC Biological and Pharmaceutical Sciences*, *10*(3), 16-22. https://doi.org/10.30574/gscbps.2020.10.3.0045