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Pharmacological Composition of *Thymus Serpyllum* and Its Components

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Abstract

Thymus serpyllum L., a member of the Lamiaceae family, thrives across Eurasia, excluding the tropics, as well as in North Africa, on the Canary Islands, and in North–West Greenland. Its aboveground parts are widely used in ethnomedicine, mainly for treating diseases related to the respiratory and gastrointestinal systems. Recently, its essential oils have become increasingly popular as an important product of plant origin. The composition of these oils depends on the geographical region, the stage of plant development, the harvest season, habitat, and climatic conditions. Thymus serpyllum essential oil has recently been used in modern medicine due to its pharmacological properties, such as antioxidant, antimicrobial, and anti-carcinogenic activity. The antioxidant and antimicrobial properties of Thymus serpyllum essential oil are attributed to its components' synergistic and cumulative action. Further research is needed to explore its potential anticancer and cytotoxic effects and enhance its cytotoxic properties for the development of corresponding medicinal preparations. Thymus serpyllum essential oil, derived from the plant traditionally used in medicine, represents a significant natural resource for the pharmaceutical industry due to its pharmacological properties. Additionally, it can serve as a source of natural antioxidants, food additives, or components for functional food products in the food industry.

Keywords: Thymus serpyllum; Flavonoids; Thyme; Antioxidant; Anticancer; Antimicrobial.
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1. Introduction

T.serpyllum L., commonly known as thyme, is a valuable aromatic plant belonging to the *Lamiaceae* family (Fig. 1). With approximately 150 species within the *Lamiaceae* family, it is widely distributed across Asia, Eurasia, Kazakhstan, and Russia. This versatile herb has become naturalized globally, notably in Eurasia, Europe, and America.[1]

T. serpyllum L., also known as Brooklyn thyme, wild thyme, or creeping thyme, is a perennial shrub native to Central Asia in the Republic of Kazakhstan: Almaty, Almaty region, Zhetysu region, Semipalatinsk, Petropavlovsk (Fig. 2). The term "*serpyllum*" traces back to a Greek word that signifies "to creep," aptly describing the plant's tendency to spread along the ground. It features an elongated stem that is woody at its foundation, culminating in a non-reproductive cluster of leaves at its peak.[2] The leaves are oval-shaped, broadening at the top and narrowing towards the base, measuring between 4–6 mm in length and 2–4 mm in width. They are smooth on both the upper surface and the underside. However, near the base, along the margins, they possess long hair-like structures known as trichomes.[3] The leaves are marked by a distinctly noticeable central vein flanked by less conspicuous side veins. Inflorescences are 4–7 cm tall and form in a series along a low-lying stem, with a uniform layer of trichomes on all sides. Flowers are located at the top of the stems and form spherical (or more rarely elongated) verticillaster. It flowers from May to September. Wild thyme grows best on dry, stony ground, open sandy heaths, and grasslands.[4]

The therapeutic qualities of *T. serpyllum* have been

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recognized and utilized in both conventional and folk medicine for decades, if not centuries. The herb, especially the top part of the plant's aerial segments harvested during flowering, is known for its curative effects, which are attributed to its rich essential oil content. In recent times, there has been a surge in research focusing on the ethnobotanical, phytochemical, and pharmacological attributes of *T. serpyllum*, highlighting its value as a premium ingredient for various pharmaceutical and chemical product formulations.[5] This herb finds application in creating natural medicinal products, including syrups, tinctures, infusions, decoctions, teas, and oils. The rise in strains of pathogens resistant to multiple drugs has spurred detailed studies into the phytochemical and pharmacological potential of *T. serpyllum*, revealing its significant antioxidant, antimicrobial, antitumor, and cytotoxic benefits. These studies support its effective use in medicine and its incorporation into pharmaceuticals, foods, and cosmetics. In addition, the increased pressure from consumers for natural products as supplements and their clinical application instead of synthetic chemicals, generally perceived by the public as more toxic, has also stimulated research into many medicinal and aromatic plants of which *T. serpyllum* occupies a very important place.[6]

2. Botanical background

2.1 Classification

(Institute of Botany and Phytointroduction. Almaty, Kazakhstan. 2024)
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Dividion: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae
Genus: Thymus L.
Species: *Thymus serpyllum* L.

2.2 Morphological studies

The stems are cylindrical or indistinctly tetrahedral, pubescent under the inflorescence with hairs located perpendicular to the pedicel or slightly downward directed. The leaves are sessile or short-stemmed, elliptical or oblong-elliptical, glabrous, with well-marked veins, 6-10 mm long. Inconspicuous dotted essential oil glands are located on the entire surface of the leaf. The leaves are ciliated along the edge to the middle or in the lower third. The flowers are small, collected in half-bunches of several pieces, forming a glabrous inflorescence or single. The calyx is narrowly bell-shaped, densely pubescent, 4-4.5 mm long. The three teeth of the upper lip of the calyx are small (the middle one is larger), acute-angled, bent, and ciliated at the edges. The corolla is 5-8 mm long (Table 1). The color of the leaves is green, and the calyx is reddish-brown. The corolla is pinkish-purple, bright pink, rarely white. The smell is fragrant, and the taste of the aqueous extract is bittersweet.

Fig. 1 Aerial parts of *T. serpyllum L. (Photo by Aitbekov R).*

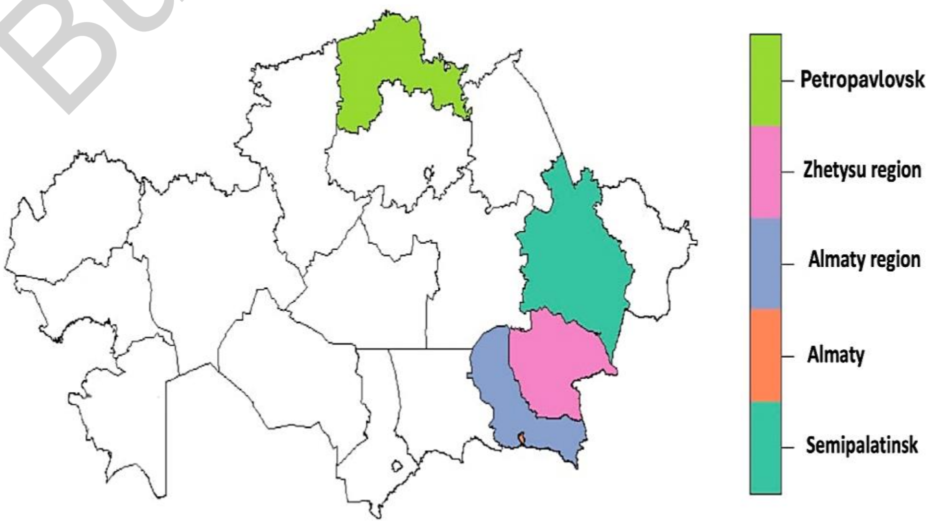

Fig. 2 Map of distribution, *T. serpyllum L. (Source: Institute of botany and Phyto introduction, Almaty, Republic of Kazakhstan)*

Table 1. Comparative characteristics of morphological features of plants *T. serpyllum* L.

Diagnostic signs	*Thymus serpyllum* L.
The cross-section of the stem	Cylindrical or vaguely tetrahedral
The nature of the pubescence of the stem	It is lowered in all parts from all sides.
The upper part	by hairs perpendicular to the stem
The middle part	
The lower part	
	
Leaf shape	Linear or narrowly elliptical

The presence of a petiole	The leaves are sessile or have a very short petiole
Leaf pubescence	Up to the middle or in the lower third, ciliated along the edge 
Inflorescence	capitate
The direction of hair growth on the pedicel	Perpendicular to the pedicel or slightly downward directed 
Cup shape	Narrow-ringed
The color of the cup	Purple
Characteristics of the teeth of the upper lip of the cup	Three teeth are small (the middle one is larger), sharp-angled, bent

In vitro micropropagation is an aseptic technique whereby small explants cultured in an artificial medium give rise to complete plants directly or indirectly. This technique is employed as a tool for ex-situ conservation. The tendency of *Thymus* to exude phenolic compounds into the culture medium makes the regeneration of tissues difficult due to the lethal browning of the medium, which results in high mortality. In vitro, browning is primarily caused by the oxidation of phenolics present in plants by a copper-containing oxidase enzyme, PPO (polyphenoloxidase), which is synthesized in an oxidation condition after tissue wounding. This results in the necrosis of cells, thereby compromising the experiment, economy, and time.

To address the issue of tissue browning in vitro, various approaches have been employed by researchers, including the addition of phenol trappers or antioxidants, such as activated charcoal, PVP (polyvinylpyrrolidone), citric acid and ascorbic acid. These have been shown to . Ascorbic acid is utilized in the present study as a means of controlling browning in vitro. The most significant plant species in the northwest Himalayas is *T. serpyllum*. It is traditionally employed as a remedy for various ailments and is renowned for its traditional applications. The current study examined the in silico and in vitro antioxidant potential of T. serpyllum essential oil. The findings revealed that thymol, a bioactive phyto compound, demonstrated considerable antioxidant potential through in silico screening. Furthermore, molecular dynamics simulation data validated the stability of the protein-ligand complex. The in vitro antioxidant activity demonstrated that the essential oil and thymol exhibited notable antioxidant efficacy comparable to that of the standard antioxidant ascorbic acid. Based on the outcomes of the in silico and in vitro experiments, it can be posited that *T. serpyllum* essential oil has the potential to serve as a potent antioxidant and functional food ingredient.[7]

2.3 Ethnobotany and the role of plants in traditional medicine

The extensive application of various *Thymus* species has historical roots tracing back to ancient Egypt, where these were utilized in the creation of fragrant ointments, in embalming rituals, and for therapeutic objectives. Similar uses were recorded in ancient Greek and Roman cultures, as evidenced by the texts of Pliny the Elder in the 1st century, Dioscorides in the 2nd century, and *Philippus Aureolus*

Theophrastus Bombastus von Hohenheim, known more widely as Paracelsus. "Thyme is known to all," began Dioscorides, the renowned physician, in his exploration of the herb's pharmacological benefits, a topic backed by over three thousand years of empirical knowledge. He detailed how thyme served as a remedy for asthma and facilitated clearing blockages in the throat and stomach.[8] Geographically, the cultivation and use of these herbs did not extend beyond the northern boundaries of the Alps until the first documented mentions of thyme's medicinal uses in the region appeared in "Physica" by Abbess Hildegard von Bingen (1098–1179) and in the writings of Albertus Magnus (1193–1280). This trend persisted into the 16th century, as evidenced by the "Herbal" of the botanist P. Mathiolus (1505–1577), which was among the first to highlight thyme's potent therapeutic effects. Since that time, a wide range of healing attributes has been ascribed to thyme, with some grounded in empirical evidence and others more contentious. The dissemination of thyme across Europe is credited to the Romans, who utilized it for room purification and to "enhance the scent and taste of cheeses and liquors." During the Middle Ages in Europe, thyme was tucked under pillows to promote restful sleep and prevent bad dreams. In this era, it was common for women to present thyme leaves to knights and soldiers, symbolizing a gift of bravery.[9] The Chilandar Medical Codex, dating from the 15th to 16th centuries, documents the medicinal application of wild thyme in treating cold-induced headaches, laryngitis, and gastrointestinal ailments and as a cough suppressant. Furthermore, in the Renaissance era, wild thyme was found to be used in the internal treatment of both malaria and epilepsy.[10] The tops of *T. serpyllum*, widely known across numerous European countries and globally, are traditionally employed for their anthelmintic, potent antiseptic, antispasmodic, carminative, deodorizing, diaphoretic, disinfecting, expectorant, sedative, and tonic properties.[10] Its primary application is in treating ailments about the digestive and respiratory systems. In the Western Balkans, the plant is notably valued for its sedative effects, enhancing blood flow, lowering cholesterol levels, and boosting the immune system.[11] In the northeastern Italian Alps, people use infusions or decoctions of the plant's aerial parts, harvested during the flowering phase, to alleviate rheumatism.[12] Research by Gairola and colleagues highlights its application in certain Indian regions for menstrual discomfort management, whereas Shinwari and Gilani document its use as an anthelmintic in Northern Pakistan. Additionally, *T. serpyllum* is found to be externally used as an antiseptic for wound care, eczema treatment, and swelling reduction.[13-19] it's a prized culinary herb in parts of Italy, especially for seasoning meats and fish. Ethnobotanical research in Catalonia and the Balearic Islands has demonstrated its use in traditional animal care, particularly against diarrhea.[20] The British Herbal Pharmacopoeia recognizes this species for its medicinal value, recommending it for conditions like bronchitis, bronchial catarrh, whooping cough, and sore throats, specifically emphasizing its effectiveness against whooping cough. This detailed study suggests its use alongside other botanicals (such as Coltsfoot, *Tussilago farfara* L., or Horehound, *Marrubium vulgare* L.).[21-24] For treating acute pharyngitis through gargling, it is advised to use it with either blackberry leaves (*Rubus fruticosus* L.) or Echinacea (*Echinacea* sp.). The Physician's Desk Reference (PDR) for Herbal Medicines notes that *T. serpyllum* is an active ingredient in several formulations designed to soothe coughs, with its alcohol extracts being key components in cough and cold remedies. The advised dosage for this herbal remedy ranges from 4 to 6 grams daily.[25-29]

Ancient medicine defined the nature of *T. serpyllum* as hot water that is II degrees dry. It kills lice and is useful for internal tumors. It is boiled in vinegar, then mixed with rose oil and lubricated with the head. This remedy improves memory and treats insanity, meningitis, lethargy, and headache. If you drink 4.5 g. His herbs and vinegar will cleanse the brain. The use of *T. serpyllum* with wine helps with cold liver tumors, expels worms, expels a dead fetus from the womb, drives urine and menstruation. It is useful for intestinal colic and the leakage of urine in drops.[30]

T. serpyllum seeds are hot and dry. They promote easy childbirth, drive menstruation, and winds. But, they are harmful to the lungs. Their dose for admission is up to 7 grams.[31]

In folk medicine of Central Asian countries, tea from *T. serpyllum* herb is drunk for intestinal colic as a carminative, anthelmintic, anticonvulsant. Externally, herbal decoctions are used as an analgesic and wound healing agent.[32]

In Russian folk medicine, infusions decoctions of *T. serpyllum* herb are used for insomnia, as an expectorant for bronchitis and tuberculosis, as a carminative diuretic. Baths in a decoction of herbs are used for metabolic disorders and rheumatism. Externally, herbal decoctions are used as a wound healing agent.[33]

In folk medicine of Belarus, an infusion of *T. serpyllum* herb is drunk for colds, coughs, nervous diseases, and rheumatism.

In Azerbaijani folk medicine, the herb is used as a carminative. An aqueous infusion of herbs with burnt sugar is drunk with poor digestion and dysentery as an anthelmintic. *T. serpyllum* herb with plantain herb is used to treat hypertension and insomnia. In the case of fainting, the herb powder is inhaled.[33]

In Armenian folk medicine, alcohol obtained from *T. serpyllum* is used for diseases of the heart, liver, diarrhea, and as an antiseptic.

In Bulgarian folk medicine, the *T. serpyllum* herb and its seeds are used for bronchial asthma and peptic ulcer disease. Externally, herbal decoctions in the form of baths are used as a good sedative for neuroses.[33]

In Austria, a fresh plant is considered a good anticonvulsant.

In Mongolian and Tibetan folk medicine, *T. serpyllum* is used as an antitussive remedy for childhood infections, anthrax, and lymphadenitis. The herb is used externally in the treatment of burns.

2.4 Biological and pharmacological activities

2.4.1 In-vitro studies

2.4.1.1 Pharmacological properties

Research into the essential oils from plants within the *Thymus* genus, including *T. serpyllum*, has demonstrated that these oils' chemical composition and yield are influenced by various factors such as geographic location, plant development stage, harvesting season, habitat, and climatic conditions. Essential oil content in *T. serpyllum* varies widely, typically ranging from 0.1% to 0.6%, though some studies report variations extending from 0.1% to 1%. Specific investigations into the yield of *T. serpyllum's* essential oil in different regions have provided insights into this variability. For instance, in Estonia, oil content varies between 0.6 and 4.4 mL/kg, with one locality specifically yielding 3 mL/kg, aligning with standards set by the European Pharmacopoeia. In Armenia, yields from five regions ranged from 4.5 to 7.4 mL/kg. Pakistani samples of wild thyme have shown yields of 0.48%, or 29 g/kg. Furthermore, in Serbia, oil yields from plants on Mt. Kopaonik and Mt. Pasjača were recorded at about 3 mL/kg (~0.3%) and 4.1 g/kg (~0.1%), respectively. These findings underline the significant variability in essential oil yields from *T. serpyllum*, which can be attributed to the 'plant's environmental and geographic conditions. Over the past two decades, a growing body of research has focused on the chemical composition of *T. serpyllum* essential oil, revealing its rich and varied makeup.[34-40] The *Thymus* genus, to which *T. serpyllum* belongs, is known for its chemical polymorphism. This means the plant can exhibit several chemotypes, including geraniol, germacrene D, citral, linalool, (E)-caryophyllene, α-terpinyl acetate, carvacrol, and thymol.[41] According to the Physician's Desk Reference (PDR) for Herbal Medicines, carvacrol is the predominant component in the essential oil of *T. serpyllum*. However, the oil is also rich in other compounds such as borneol, isobutyl acetate, caryophyllene, 1,8-cineole, citral, citronellal, citronellol, p-cymene, geraniol, linalool, α-pinene, γ-terpinene, α-terpineol, terpinyl acetate, and thymol, all present in significant concentrations.[42] Notably, are both monoterpenic phenols, and both carvacrol and thymol are recognized for their potent antiseptic properties. They are rapidly absorbed upon application and quickly metabolized, bypassing the first the biotransformation phase. Instead, they directly undergo conjugation with sulfuric and glucuronic acids. These compounds are predominantly excreted in the urine within 24 hours, mainly as conjugates, and less frequently in their unchanged forms.[43] The European Pharmacopoeia stipulates that *T. serpyllum* herb must contain at least 1.2% essential oil, with carvacrol and thymol combined accounting for no less than 40% of the oil.[44] Beyond these essential oils, wild thyme is also a source of flavonoids, phenolic carboxylic acids and their derivatives, triterpenes, and tannins.[45] Further research by Kulišić *et al.* highlights γ-terpinene and p-cymene as major components of the essential oil.[46] This expanding knowledge base deepens our understanding of T. serpyllum's pharmacological potential and enhances its prospective uses in both traditional and modern medicine. The ongoing study of its chemical diversity underscores botanical medicine's complexity and the necessity for thorough, multidisciplinary research approaches. Research into the chemical composition and concentrations of *T. serpyllum* essential oil has shown considerable regional variations globally. For instance, oil content in wild thyme from Russia's Altai Mountains ranges from 0.5% to 1%, with composition notably differing by altitude. At 150 meters above sea level in the village of Kolyvan, the oil primarily comprises *β*-myrcene (4.0%), p-cymol (3.8%), 1,8-cineole (14.0%), cis-*β*-terpineol (8.2%), camphor (4.0%), and trans-nerolidol (29.8%). Meanwhile, higher up in the village of Mendur-Sokkon (500–750 m a.s.l.), the oil is rich in p-cymol (14.5%), 1,8-cineole (5.6%), *γ*-terpinene (17.2%), and carvacrol (29.6%), with thymol content below 2% in both locations.[47] In Lithuania, neither thymol nor carvacrol was detected in the wild thyme's oil,[48] diverging from its typical profile where these components are dominant, as also observed in Estonia.[49] Contrastingly, the main constituents of the oil from Serbia's Mt. Kopaonik include trans-caryophyllene (27.7%), *γ*-muurolene (10.5%), and *α*-humulene (7.5%).[50] On Mt. Pasjača, however, the oil predominantly contains trans-nerolidol (24.2%), germacrene D (16.0%), thymol (7.3%), *δ*-cadinene (3.7%), and *β*-bisabolene (3.3%).[51] In Pakistan, thymol (53.3%) and carvacrol (10.4%) chiefly characterize the oil,[52] while a study from Pakistan's Gilgit Valley found it dominated by carvacrol (44.4%) and o-cymene (14.0%).[53] Furthermore, De Lisi *et al*. noted compositional variations among southern Italian ecotypes, with geraniol being most concentrated in biotypes S2 and S3 (35% and 22%, respectively), whereas biotype S1 was rich in thymol (32.6%).[54] In a Lithuanian study, Ložienė *et al*. analyzed 26 samples from 14 habitats, identifying five chemotypes including 1,8-cineole, germacrene B, (E)-*β*-ocimene, *α*-cadinol, and cis-p-ment-2-en-1-ol, pointing to significant diversity in oil composition even within the same region.[55] The varying composition of *T. serpyllum* essential oil across different regions underscores its unreliable nature as a chemotaxonomic marker. Nevertheless, its composition's medical, cosmetic, and industrial significance, particularly the phenolic monoterpenoids like thymol and carvacrol, known for their antimicrobial properties and inhibition of lipid peroxidation, remains high. These elements serve as potent antimicrobials and natural antioxidants, playing a crucial role in the metabolic response to oxidative stress induced by environmental factors or pathogenic organisms.[56]

It is known that from a pharmacological perspective, the plant has antibacterial potential against various clinical and other strains of both gram-positive and gram-negative bacteria.[57]

2.4.1.2 Bioactive compounds of *T. serpyllum*

The leaves and flowers of *T. serpyllum* contain a diverse array of phytochemicals that have been extensively identified. These include essential oils (such as tricyclene, sabine acetate, α-terminene), alkaloids, carbohydrates, essential fatty acids, glycosides (such as cardiac glycosides, flavonoid glycosides, saponins), phenolic compounds (like phenylpropanoids, flavonoids, tannins), phenolic carboxylic acids, polyacetylenes, steroids, terpenoids (for instance, ursolic acid, oleanolic acid, dihydrosolic acid), carotenoids, mineral elements, diterpenoids, and aqueous extracts rich in flavonoids, particularly rosmarinic acid and luteolin-7-glucoside.[57] Table 2 shows the chemical composition of *T. serpyllum.* Most of these phytochemicals have been obtained using various extraction methods, including aromatic oil extraction, alcohol extraction, aqueous extraction, butanol fractionation and infusion preparation. More than 110 components have been identified in the essential oil obtained from the aboveground parts of *T. serpyllum.*

Table 2. The chemical composition of *T. serpyllum.*

Plant part	Dedicated connections	References
Essential oil		
The aboveground part	Essential oil, up to 4.4%:	57-60
	•Tricyclene •α-Tuyon •β-thujón •α-pinene •β-Pinekamphene •Sabinen •Sabinyl Acetate •cis-sabinene hydrate •Trans-sabinene hydrate •cis-sabinol •Trans-Sabinol •1-octene-3-ol •β-Myrcene •α-Phellandrene •bicycyclesquifellandren •3-karen •α-Terpinene •γ-Terpinene •Terpinolene •N-Cymol •limonene •1,8-Cineole •trans-β-ocimen •cis-β-ocimen •alloocymen •cis-β-terpineol •trans-β-terpineol •α-Terpineol •linalool •linolyl acetate •Cis-Linalool Oxide •cis-n-ment-2-en-1-ol •Pinocarveoli •camphor •Borneol •Isoborneol •m-ment-1-en-8-ol •Terpineol-4 •Terpinyl Acetate •thymol methyl ester •Pulegón	

•Carvacrol Methyl Ester
•cis-citral
•Citronellol
•Citronellal
•Citronellyl Acetate
•Geraniol
•geranyl acetate
•Geranylbutyrate
•Geronial
•Trans-Citral
•1-Decanol bornyl acetate
•isobornyl acetate
•thymol
•Carvacrol
•Eucalyptol
•α-Copen
•β-Bourbonene
•β-Elemen
•γ-elemen
•δ-elemen
•Caryophyllene
•Caryophyllene oxide
•Humulene
•Humulene epoxide
•Alloaromadendrin
•γ-Muurolen
•α-Muurolen
•T-Muurol
•cis-muurol-5-en-4-β-ol
•Germacrene-D
•Bicyclogermacrene
•β-Bizabolen
•Kadina-1,4-diene
•γ-Kadien
•α-Kadinen
•δ-Kadinen
•Endo-1-bourbonenol
•cis-α-bizabolene
•Bisabolol
•α-bisabolol
•β-bisabolol
•Trans-nerolidol
•Germacradienol
•germacrene V
•germacrene -D-4-ol
•Germacrene D
•Bicyclogermacrene
•Spatulenol
•Viridifluorol
•Ice Maker
•Neral
•Carvon
•cis-dihydrocarvone
•Farnesen
•Zingiberen

	•Italizen	
	•Selina-3,7(11)-diene	
	•1,10-epicubenol	
	•Chedicariol	
	•Verbenol	
	•cis-verbenol	
	•trans-verbenol	
	•Elemol	
Phenolic compounds		
	Phenylpropanoids:	61-67
	• thymol	
	• carvacrol	
	• methylcarvacrol	
	• isoevgenol	
	Phenolic Carboxylic acids:	68-74
	• coffee 0.02%	
	• 5-caffeoylquine 0.05%	
	• gallic rosemary 0.83%	
	Flavonoids: 2.86%	75
	• apigenin	
	• luteolin	
	• quercetin	
	• diosmetin	
	• scutellarein	
	• Apigenin-7-glucoside	
	• Apigenin-4'-O-β-D-n-coumaroyl-glucoside	
	• Diosmetin-7-O-β-D-glucuronide	
	• luteolin-galactoarabinoside	
	• Luteolin-7-O-β-D-glucoside	
	• Luteolin-7-O-β-D-diglucoside	
	• scutellarein-glucosylglucuronide	
	• eriocitrin	
	•Tannins 3.32%	75
Other classes of natural plant compounds		
	Triterpenoids:	76-80
	• ursolic acid	
	• oleanolic acid	
	• dihydro ursolic acid	
	• 3-β-hydroxyomane-12-en-28-ova acid	
	Benzene and its derivatives:	81-85
	• 1-methyl-3-(1-methylethyl) benzene	
	• 1-methoxy-4-methyl-2-(1-methyl) benzene	
	• 2-methyl-5-(1-methylethenyl) phenol	86
	Steroids:	87-89
	• Sitosterol-3-0-β-D-Glucopyranoside	
	Ascorbic acid 0.14%	90
	Carotenoids 0.11%	91
	Aliphaticheskyye uglyph:	92
	•n-geptadecan	

• n-nonadecan	
• N-Gen mwcozan	
• 7,11-dimethyl-1,6,10-dodecatriene	
• 2-geptenol	
• 3-octanol	
• 1-octen-3-ol	
• 1-decanol	
• 1-dodecanol	
•3-octanone	
• 27-ketotriacontanol	
Higher fatty acids:	93
• 3-ketopentatriacontane	
Macronutrients:	94
• Potassium – 26.1	
• Calcium – 12.2	
• Magnesium – 3.9	
• Iron – 0.95 Trace Elements:	
• Manganese – 0.31	
• Copper – 0.48	
• Zinc – 0.48	
• Cobalt – 0.12	
• Molybdenum – 64.0	
• Chrome – 0.1	
• Aluminum – 0.66	
• Barium – 0.58	
• Vanadium – 0.35	
• Selenium – 7.1	
• Nickel – 0.2	
• Strontium – 0.36	
• Lead – 0.13	
• Boron – 108.4	
Fatty oil 33.6% Fatty acids:	95-97
• palmitic (2.6%)	
• stearic (2.3%)	
• oleic (11.4%)	
• linoleic (20.9%)	
• linolenic (62.1%)	

Galovicova *et al*. isolated essential oil from thyme grass and established its physico-chemical properties. The essential oil they obtained had a specific gravity of about 0.9, a refractive index from 1.490 to 1.515, a color from light yellow to intense orange, with a characteristic odor indicating the presence of thymol and carvacrol.[98]

The amount of essential oil in creeping *T. serpyllum* ranges from 0.47% to 4.40%. The main components of *T. serpyllum* essential oil are thymol and carvacrol. The phenol content determined by the GC method in various samples of creeping thyme was: thymol 0.212-1.013%, carvacrol 0.086-1.403%, the number of phenols 0.374-1.615%, which is why thymol prevails in some samples, carvacrol in others.

The structure of the main flavonoids and terpenes allocated from *T. serpyllum* is shown in Figs. 3 and 4, accordingly. Most phytochemicals informed from *T. serpyllum* have been separated from its aromatic oil, alcoholic extraction, aqueous extraction, butanol fraction, and infusion preparation.

The primary constituents of the oil extracted from *T. serpyllum* encompass tricyclene, sabinene, sabinyl acetate, terpinolene, limonene, alloocymene, linalool, borneol, and pulegone.[99] Flavonoids, notably rosmarinic acid and luteolin-7-O-β-D-glucoside, are notably abundant in both alcoholic and aqueous extracts of *T. serpyllum*. Additionally, phenol carboxylic acids like caffeic acid, 5-caffeoylquinic acid, and gallic acid have been detected in the ethanol extract of *T. serpyllum*.[100] Various flavonoids, including apigenin acid, luteolin, quercetin, diosmetin, scutellarein, apigenin-7-glucoside, rosmarinic acid, rutin, alongside volatile components like borneol and cineole, have been identified in infusions prepared from *T. serpyllum*.[101] Among these, apigenin acid and rosmarinic acid are the most abundant flavonoids in *T. serpyllum* infusions, followed by rutin, scutellarein-glucosyl-glucuronide, and eriocitrin.[102] The

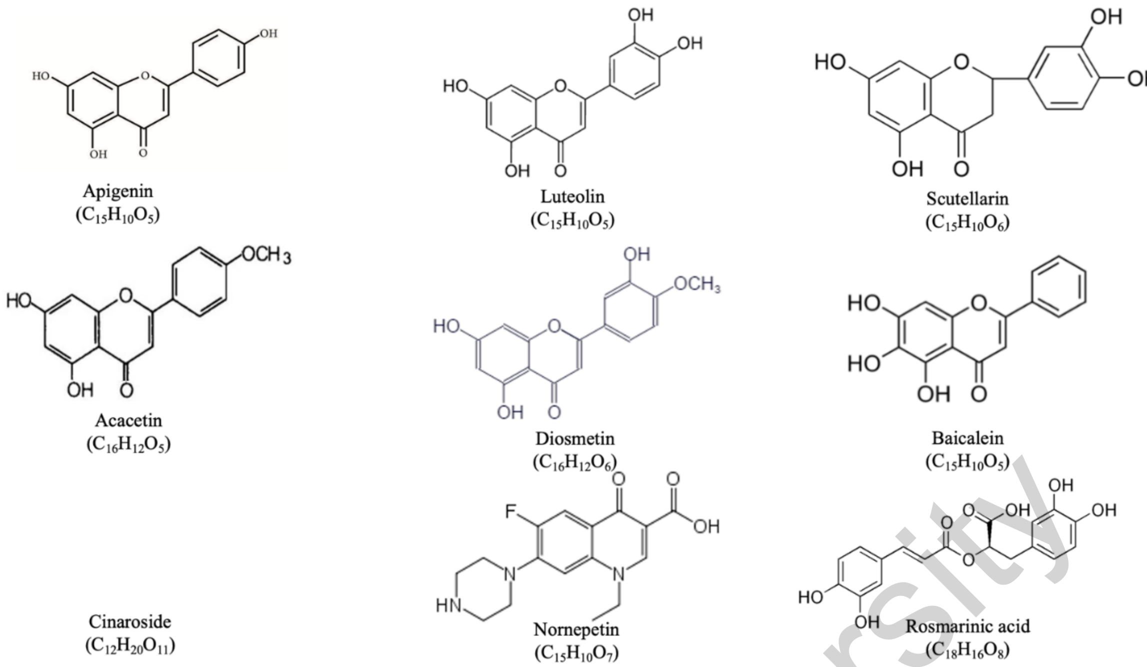

Fig. 3 Structure of main flavonoids isolated from *T. serpyllum.*

predominant carbohydrates documented in this plant are xylose, glucose, galactose, fructose, uronic acid, and cellulose acetate.[103] Comparison of phytochemicals in the flowers and leaves of *T. serpyllum* reveals distinct compositions. Ursolic acid stands out as the primary phytochemical present in flowers, while the leaves exhibit higher concentrations of timolol (a non-cardioselective beta-blocker), isoeugenol, methylcarvacrol, and carvacrol.[104] However, it's crucial to consider that, similar to other plants within this botanical family, the chemical makeup of *T. serpyllum* can vary significantly based on environmental factors such as climate, water availability, altitude, and soil composition.[105]

2.4.1.3 Anticancer and antimutagenic effects

The potential antitumor activity of *T. serpyllum* has been extensively studied across various cancer cell lines and animal cancer models. However, it's crucial to note that while numerous studies have investigated its antitumor effects, most of these investigations relied on in vitro experiments [105] with relatively few conducted in vivo.[106] Notably, there is a lack of evidence in the literature regarding the impact of extracts derived from crude *T. serpyllum* on tumor cells. The potential

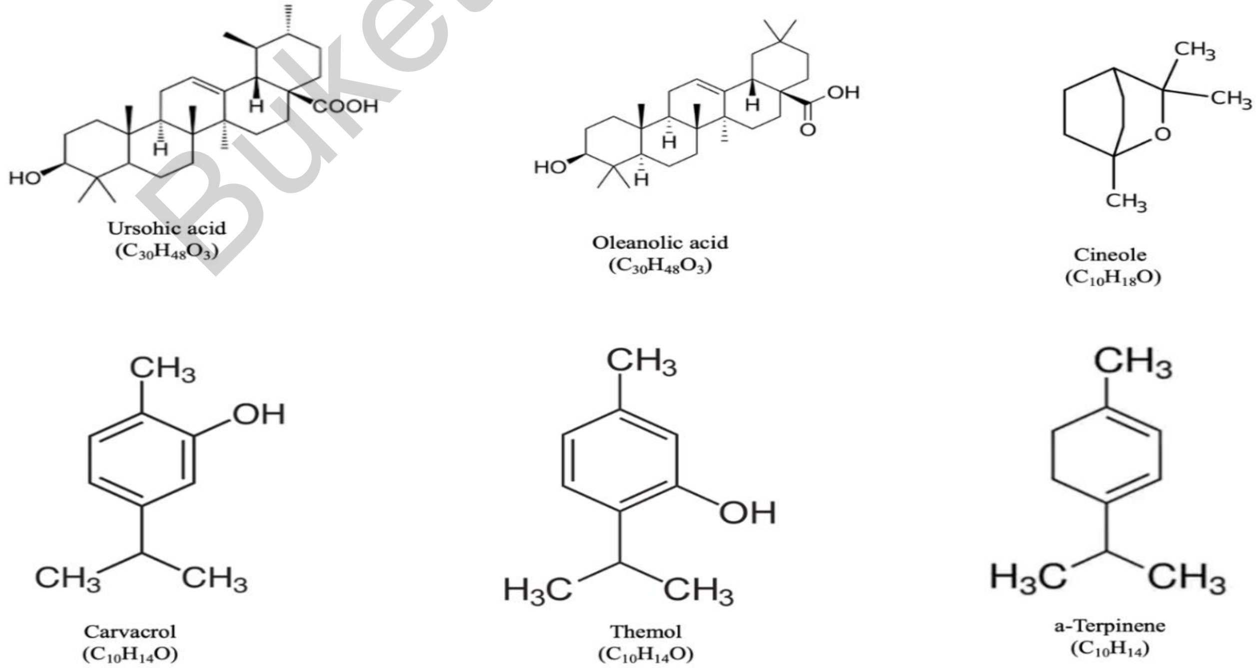

Fig. 4 Structure of main terpenes and terpenoids isolated from *T. serpyllum.*

antitumor activity of *T. serpyllum* has been extensively studied across various cancer cell lines and animal cancer models. However, it's crucial to note that while numerous studies have investigated its antitumor effects, most of these investigations relied on in vitro experiments[107] with relatively few conducted *in vivo*.[108] Notably, there is a lack of evidence from the literature regarding the impact of extracts derived from crude *T. serpyllum* on tumor cells. Furthermore, a study by Mihailovic *et al*. demonstrated significant *in vivo* anti-cancer activity of thyme genus representatives, including *T. serpyllum*.[109] However, despite the close relation between these species, there were variations in their specific effects. The differences observed in the volume of neoplasm and the nature of structural changes in the tumors in animals between the two experiments could potentially be attributed to the varying susceptibility of cancer cells in vivo to phytochemicals, the volatility of the chemical composition of the analyzed plant species, as well as dosage factors.[110]

One of the primary constituents of aromatic thyme oil, carvone, exhibits significant cytotoxic effects on tumor cells. Studies have verified that the compound carvacrol demonstrates substantial cytotoxic activity against leukemia P388 in laboratory mice and hepatitis-2.[111] However, timolol (a non-selective beta-blocker), also among the main components of essential oils derived from various types of thyme, may hinder the effective expansion of pulp fibroblasts. When comparing the antitumor activity of essential oils from the most prevalent types of thyme in the growth of four human tumor cells, it has been proven that the aromatic oil of T. serpyllum exhibits the greatest antitumor activity.[112] In a significant study conducted in 2014, among 21 isolated compounds—including carvacrol, thymol, and thymoquinone, which are the principal components of the extract of essential oil from creeping thyme—it was established that this hexane extract is cytotoxic for six types of cancer cells (MDA-MB-231, MCF-7, HepG2, HCT-116, PC3, and A549). This extract demonstrated superior antitumor activity with HepG2 (liver carcinomas), followed by HCT116 (colon cancer), MCF-7 and MDA-MB-231 (breast cancer), PC3 (prostate cancer), and A549 (lung carcinoma).[113]

T.serpyllum contains various chemical compounds, including carvacrol and thymol, which have been observed to possess strong cytotoxic activity. This makes them useful in the treatment of cancerous cells. Carvacrol is a monoterpene phenol that has been identified as a potential inhibitor of cancer development. It has also been shown to have an anticancer effect by limiting cell proliferation and preventing metastasis in DEN-induced hepatocellular carcinogenesis.[114]

2.4.1.4 Antioxidant activities

Oxidative stress is recognized as a pivotal factor in the onset and progression of numerous diseases, notably cancers, cardiovascular disorders, diabetes, and neurological conditions.[114] A comprehensive pharmacological screening of six types of *T. serpyllum* was conducted, comparing them with creeping thyme. This screening led to the identification of expectorant, anti-inflammatory, and angioprotective activities for the first time. The antibacterial properties of infusions and essential oils derived from these plants were also investigated, while the antioxidant activity of water-alcohol extracts was experimentally confirmed.[115] Particularly noteworthy was the robust antioxidant activity observed in extracts produced with 70% and 50% ethyl alcohol, attributed to the presence of phenolic compounds, including flavonoids and phenolic carboxylic acids, within the thyme genus plants.[116] Hussain *et al*. also established that the essential oil of *T. serpyllum* growing in Pakistan exhibited less ability to neutralize DPPH radicals than BHT and thymol.[117] Their investigation, evaluating the reaction with the stable free radical diphenylpicrylhydrazyl through spectrophotometric analysis at 517 nm, revealed that aqueous extracts exhibited maximum antioxidant activity, ranging from 0.02 mg/ml to 0.14 mg/ml.[118] The antioxidant capability of creeping thyme in the middle taiga subzone of the Komi Republic was comparable to that of natural populations in Portugal (0.032 mg/ml) and significantly higher than plants grown in Croatia (0.45 mg/ml). Subsequently, the researchers established a correlation between antioxidant activity and the content of flavonoids,[119] affirming that about 50% of the antioxidant capacity is linked to flavonoid content. This aligns with existing literature indicating that the antioxidant potency of aqueous extracts from thyme plants is attributed to their high flavonoid levels, such as luteolin-7-glucoside and apigenin-7-glucoside.[120] Additionally, approximately 37-25% of the antioxidant activity may be associated with other phenolic compounds, predominantly phenolic carboxylic acids.

Due to the presence of rosemary acid, flavonoids, thyme preparations, essential oils have a pronounced antioxidant, anti-inflammatory, and immunomodulatory effect.[120]

Thymoquinone, a constituent of the plant *Thymus*, has been demonstrated to exert antioxidant effects in brain tissue in vivo. Furthermore, a hexane extract of the plant has been shown to possess anticancerous potential against six cell lines, namely HepG2, MCF-7, MDA-MB-231, PC3, HCT-116, and A549.[121]

2.4.1.5 Antibacterial effect

The antibacterial activity of infusions and essential oil extracted from thyme herbs was investigated. The evaluation of antimicrobial activity involved applying these studied infusions and essential oil onto a nutrient medium, followed by seeding microorganisms onto the agar surface.[122-126] Several strains of microorganisms were employed for this study, including *Pseudomonas aeruginosa, Staphylococcus aureus, Candida fungi, Escherichia coli, Bacillus cereus*, and *Proteus vulgaris*. The microbial load used was 100 million microbial bodies in 1.0 ml.

During the assessment of the antimicrobial activity of the obtained infusions and extracted essential oil, a range of dilutions were prepared at various concentrations in sterile nutrient agar cooled to 50 °C. Following mixing, the diluted contents were poured into sterile Petri dishes and left at room temperature to solidify.[127] Subsequently, the Petri dishes were divided into sectors, each of which was inoculated with suspensions of daily crops using the streak method, containing 100 million microbial bodies in 1.0 ml. As a control, the same bacteria were cultured on nutrient media that did not contain the studied infusions and essential oil. These cultures were incubated in a thermostat at a temperature of +37 °C for 24 hours and 48 hours (for *Candida fungi*). After incubation, the growth intensity of microbial colonies (strong growth, weak growth) or lack of growth was assessed.[128]

In modern scientific medicine, the medicinal properties of thyme are associated with thymol contained in its essential oil.[129] Thymol is used in modern medicine as a disinfectant. Plant extracts have a pronounced antibacterial effect.[130] It also kills tapeworms. Thymol is included in the composition of special candies. Thyme essential oil has pronounced antifungal properties and is effective in treating candidiasis.[131] Several essential oils have been demonstrated to possess antibacterial and antiviral properties in various in vitro models. The oils have been tested against a range of pathogenic and non-pathogenic organisms.

Many studies have evaluated the antimicrobial activity of *T. serpyllum* essential oils, both individually and in combination with other components, including other essential oils. These studies have employed a range of bioassays, including agar well/disk diffusion and broth dilution methods, to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).[132]

In these studies, the tested concentrations varied between 5 and 15 µL, demonstrating efficacy against a diverse range of microorganisms in the presence of *T. serpyllum* essential oils. For example, the antibacterial activity with MIC (0.0625; 0.125% v/v) against *Escherichia coli* and *Staphylococcus aureus*.[133] and the MIC (0.012–0.2%) and MBC (0.012–0.2%)[133] were investigated.

The *Pseudomonas aeruginosa* strain demonstrated slightly heightened sensitivity to the essential oils of *T. vulgaris* and *T. serpyllum* (minimum inhibitory concentration (MIC) 0.5 mg/mL). The antimicrobial activity against a wide range of microorganisms was attributed to the high content of aromatic monoterpenoids, namely thymol. In a separate study, the essential oil of *T. serpyllum* demonstrated MIC/MBC (3.125–6.25/12.5 µL/mL) levels against a gram-negative bacterial strain. *T. serpyllum* demonstrated antimicrobial activity against oral pathogens, suggesting its potential use in oral care. Its MIC (2.5–5 µg/mL) and MBC (5–10 µg/mL) for bacteria and MIC (1–2 µg/mL) and MFC (2–4 µg/mL) for fungi warrant further investigation. The plant population of *T. serpyllum*, situated in sunny meadow conditions, may possess a considerable quantity of the aromatic monoterpenes carvacrol and p-cymene, and has demonstrated a broader spectrum of antimicrobial activity.

The secondary essential oils (hydrosolic essential oil fraction) of *T. serpyllum* were also found to exhibit enhanced antibacterial and antifungal activities, and they were richer in phenolic compounds (92.3%) compared to their content in primary essential oils (water-insoluble essential oil fraction after steam-distillation) (42.1%).[134] The N-propyl rosmarinate isolate from *T. serpyllum* demonstrated the most potent antibacterial activity compared to other isolates. Additionally, the *T. serpyllum* essential oil in the vapor phase exhibited antibacterial activity (33–350 µL/L), although it was less effective than other essential oils investigated in the study.[135]

2.4.1.6 Expectorant effect

The expectorant effect was examined using a model that assessed the atrial epithelium's motor function in frogs' esophagus.[136] In this experiment, frogs were positioned on a cortical plate with their bellies facing upward. Infusions from raw thyme plants, prepared at a concentration of 1:10, were applied to the tip of the frog's tongue in a volume of 0.1 ml. An infusion of the official type, specifically the creeping thyme herb, served as the comparison drug.

To measure the cilia movement of the ciliated epithelium, a 15.0 mm silk thread was placed at the base of the tongue 30 seconds after applying the studied infusions. The time taken for the thread to be ingested was recorded. Concurrently, the time elapsed during the thread movement over 10.0 mm without any infusion (control) and after the application of the studied infusion was noted. Considering the notable variability in ciliated epithelium movement among different animals, we utilized the acceleration coefficient (CU). This coefficient was calculated by determining the ratio of the speed observed after applying the studied infusion to the initial speed.[137]

2.4.1.7 Toxicological studies

In numerous clinical trials, it has been reported that the *T. serpyllum* plant generally does not cause serious side effects.[138] However, in cases of prolonged use or excessive dosage of ethanolic extract and ethereal oil of *T. serpyllum* (typically involving 12 g of the foliage), some adverse effects may manifest, including nausea, increased salivation (sialosis), cardiovascular issues, dizziness, lowered body temperature (fever reduction), allergic reactions, lividity, and even convulsions.[132] The anticonvulsant effect associated with *T.serpyllum* oil is attributed to its prolonged impact on nerves, particularly at doses exceeding 0.3 g/kg.[139] Compounds such as camphoric, borneol, and isoborneol ketone are identified as the main harmful constituents in *T.serpyllum*.[140] These compounds may induce toxic effects on embryos and infants. Consequently, the use of *T. serpyllum* is not recommended during pregnancy and lactation. Animal studies have revealed that the LD50 of T. serpyllum oil (with oral administration) and the ethanol extract (with intraperitoneal administration) is 2.5 g/kg and 4.5 g/kg, respectively.[141] Reports indicate that the potency of *T. serpyllum* can exacerbate C1-induced hepatotoxicity in laboratory mice. However, several clinical studies have not reported any hepatotoxic effects associated with this plant.[142]

The plant is non-toxic, and to prepare infusions, teaspoon of the herb is poured into a glass of boiling water. The plant should be used carefully with low blood pressure. With prolonged use, it can lead to hypofunction of the thyroid gland.[143] In general, this is a very useful and promising plant. Thyme is used for food in the countries where it grows. In addition, the herb is used in the beverage and food industries.[144]

2.4.2 Pre-clinical (in-vivo) studies

2.4.2.1 Cardiovascular disease and metabolic syndrome

Several preclinical studies have been published on cardiovascular diseases, including studies on the antidiabetic,[145] antihyperlipidemic, and liver enzyme protective activities[146] of the aqueous extract of *T. serpyllum*. The ether and aqueous extracts were observed to reduce blood glucose levels in diabetic rabbits (500 mg/kg b.w. with glibenclamide and acarbose employed as controls), with the aqueous extract demonstrating the ability to inhibit the rise in glucose levels observed in the oral tolerance test.[147] The extract demonstrated a synergistic effect with varying insulin levels, resulting in a reduction in HbA1c levels and an increase in hemoglobin levels over a three-month period.[148]

A further study conducted by the same research group in the same setting treated diabetic rabbits with a single high dose of 500 mg/kg of the aqueous extract. Blood samples were collected on days 0 and 30. The extract has been demonstrated to diminish the concentration of serum cholesterol, triglyceride, LDL, VLDL, alkaline phosphatase and transaminases while leaving the HDL level unaltered. The total cholesterol/HDL ratio was significantly reduced compared to the diabetic control group.[149] It is unlikely that these findings will have any therapeutic relevance. This is due to the high dosage tested in this study. For example, if we compare the rabbit dose in mg/kg to the human dose in mg/kg, we can see that the former is considerably higher. According to the conversion factor of 500/3.1, the human equivalent dose would be 161 mg/kg x 70 kg (human body weight), which implies a single dose of 11.3 g. This renders the study conducted by Alamgeer *et al*. implausible.[150]

A diabetic model of BALB/c mice was employed, wherein the mice were fed a high-fat diet and received two streptozotocin injections intraperitoneally. The mice were administered an aqueous extract of *T. serpyllum* at doses of 500 and 800 mg/kg/d for a period of four weeks. The treatment was found to be significantly efficacious in controlling hyperglycemia and improving glucose and insulin tolerance. Upregulated mRNA levels of the AMPK, IRS1, and GLUT2 genes were identified within isolated liver tissue. Furthermore, the liver, kidney, and pancreas cellular morphology was restored, as evidenced by histopathological examination.[151] Nanoparticles of *T. serpyllum* (10 mg/kg) have also demonstrated robust antidiabetic efficacy in streptozocin-induced diabetic BALB/c mouse models. This is evidenced by the augmentation of AMPK and IRS-1 expressions, which consequently elevate cellular glucose uptake.[152]

Additionally, the aqueous extract was demonstrated to possess antihypertensive properties and reduce vascular resistance in hypertensive and normotensive rats (100 mg/kg b.w. administered intravenously in 0.2 saline).[153] This inverse correlation between vascular resistance and plasma heme oxygenase-1 was attributed to the endogenous vasodilator carbon monoxide generated by heme oxidation, which could account for the normalization of blood pressure.[154] The antihypertensive effect of the aqueous extract of *T. serpyllum* was elucidated through the administration of an injection of 100 mg/kg b.w. as an intravenous bolus in 0.2 mL saline, which resulted in a reduction of systolic and diastolic blood pressure and total peripheral resistance in spontaneously hypertensive rats (SHR) without any observable effects on these parameters in normotensive Wistar rats. The findings indicate that the aqueous extract may offer protection against hypertension in the experimental model of essential hypertension, while the cardiac index remained unaltered throughout the treatment period in all experimental rats.[155]

2.4.2.2 Anti-inflammatory properties

As previously mentioned, thyme plants have been extensively used in folk medicine for their anti-inflammatory properties, particularly in treating upper respiratory tract diseases. Our analysis of the chemical composition of these plants revealed the presence of various compounds such as phenylpropanoids - thymol and carvacrol, phenolic compounds (including flavonoids, phenol-carboxylic acids, and coumarins), essential oils, and polysaccharides. Literature confirms that these identified natural compound classes possess anti-inflammatory activity.[156] To assess the anti-inflammatory activity, we conducted studies to observe the effects of the test infusions on exudation and proliferation processes.[157] The anti-exudative activity was examined in mice of both sexes weighing between 18.0-and 20.0 g. Acute aseptic inflammation of the animals' limbs was induced by subplantar administration of 0.1 ml of 2.5% formalin mortar.[158] The experimental group of mice received intragastric administrations of the studied infusions at specific time intervals: 2 hours before formalin introduction and 5 hours and 18 hours after its administration. The infusions used during the experiment were administered at a dose of 1000 mg/kg (in terms of dry raw materials) in a volume equivalent to 10.0 ml/kg, prepared in a 1:10 ratio. The control group of mice received purified water, mirroring the experimental group's conditions. The comparative drug used was an infusion of *T. serpyllum* herb. After 24 hours from the formalin solution administration, we evaluated the antioxidative effect by measuring the difference in mass between the edematous and non-edematous paws of the animal's hind legs, which were then amputated at the talocrural joint level.[159] The impact of the infusions on the proliferative processes of inflammation was examined using the "cotton granuloma" model.[160] This involved the implantation of sterile cotton balls weighing 25.0 mg beneath the skin in the back area. The experiments were conducted on white rats weighing between 180.0 and 200.0 g. Rats in the experimental groups received intragastric administrations of the studied infusions twice a day for seven days. The infusions, prepared at a concentration of 1:10, were administered at a dosage of 1000.0 mg/kg (in terms of dry raw materials) in a volume equivalent to 10.0 ml/kg. Rats in the control group were similarly treated with purified water. The comparative drug used was an infusion of *T. serpyllum* herb (official type). The cotton ball containing the formed granulation tissue was removed and dried after the seven-day experiment until a constant mass was achieved. The difference between the mass of the wet and dry granuloma was measured to determine the mass of the developed fibrous-granulation tissue.

2.4.3 Clinical studies

2.4.3.1 Gut, digestive, and liver disorders

A recent human pilot study was conducted to investigate the effects of an aqueous *T. serpyllum* herbal extract on gut health. In a randomized controlled clinical trial with 40 overweight subjects (N=2×20) affected by functional gastrointestinal disorders (FGIDs), referred to as gut-brain interaction disorders, 600 mg of aqueous *T. serpyllum* herbal extract was administered before breakfast for eight weeks.[161] The findings of the study suggest that wild thyme has the potential to alleviate gastrointestinal symptoms and increase stool frequency. This also resulted in an improved quality of life. A high FirmicutesA high Firmicutes characterised the stool microbiome of the study group characterised the stool microbiome of the study group to Bacteroidetes ratio, which the intake of aqueous T. serpyllum herbal extract could positively influence.[161]

3. Conclusion

Presently, there is widespread interest in traditional medicine and herbal-based treatments within the global medical community, offering promising avenues for potential new drugs. Consequently, numerous experimental and clinical studies are underway involving medicinal plants, including investigations targeting cancer cells. It is crucial to consolidate and update the results gathered from these studies. This article attempts to synthesize and discuss the available pharmacological data consistently reported on *T. serpyllum*. Based on accessible published evidence, *T. serpyllum* exhibits a spectrum of pharmacological properties encompassing antitumor, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, hypoglycemic, hypolipidemic effects, as well as positive impacts on the upper respiratory tract. Clinical trials have confirmed the effectiveness of *T. serpyllum* in acting as an antinociceptive agent and a hypolipidemic agent and in its beneficial effects on upper respiratory tract issues. Additionally, beyond these documented effects, several other biological benefits have been attributed to *T. serpyllum* in the literature, including its efficacy in treating skin diseases. However, further elucidation is required regarding the precise therapeutic applications associated with *T. serpyllum's* effects, warranting future research endeavors. Furthermore, it is imperative to conduct in-depth investigations to unravel the precise molecular mechanisms responsible for T. *serpyllum's* influence, its toxicity profile, and potential drug interactions.

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Conflict of Interest
There is no conflict of interest.

Supporting Information
Not applicable.

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