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Research article

A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of *Thymus vulgaris* Linn.

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ABSTRACT

Thymus vulgaris Linn. is a medicinal and culinary herb from the Southern European region known for its anti-infective, cardioprotective, gastroprotective, anti-inflammatory, and immunomodulatory activities since the Egyptian era. The reported pharmacological activities of *T. vulgaris* L. include antibacterial, antioxidant, anti-inflammatory, antiviral, and anti-cancerous activities. In this review, a comprehensive approach is put forth to scrutinize and report the available data on phytochemistry, ethnopharmacology, pharmacology, and toxicology of the plant. The different extracts and essential oil obtained from the plant have been assessed and reported to treat ailments like microbial infections, inflammation, non-communicable diseases like cancer, and sexually transmitted diseases like HIV-1 and Herpes. The literature review has also indicated the use of volatile oils, phenolic acids, terpenoids, flavonoids, saponins, steroids, tannins, alkaloids, and polysaccharides in pharmacotherapy. Applications of these compounds including antidiabetic, anti-Alzheimer's, cardio, neuro and hepatoprotective, anti-osteoporosis, sedative, immunomodulatory, antioxidant, anti-tyrosinase, antispasmodic, antinociceptive, gastroprotective, anticonvulsant, antihypertensive, antidepressant, anti-amnesia, and anti-helminthic activities have been mentioned. Further, based on research gaps, recommendations have been provided to evaluate *T. vulgaris* L. systematically to develop plant-based drugs, nutraceuticals, and to evaluate their clinical efficiency and safety.

1. Introduction

T. vulgaris L. or thyme, known as "garden thyme" is an aromatic and perennial flowering plant belonging to the *Lamiaceae* family [1, 2]. The Greek form of the word 'thyme' depicts 'to fumigate', owing to its use as incense or for its balsamic odour or it belonged to the class of sweet-smelling herbs [1]. Being native to Southern Europe, *T. vulgaris* L. is reported to have a worldwide distribution [2]. The plant grows well in an arid climate and unshaded areas in coarse, rough, and well-drained soil that is generally unsuitable for many plants. It appears as a short and bushy plant with several small flowers [3]. It is usually grown for commercial purposes in several countries for its dried leaves, plant extracts, plant oil, and oleoresins [4, 5]. *T. vulgaris* L. is commercially used as aflavouring agent in food industries due to its extensive aromaticity [1]. It is also used for preserving meat, chicken, and fish [6], along with its use for flowering and ornamental purposes [7]. In addition, the perfumery and cosmetic industries also use *T. vulgaris* L. for its characteristic aroma [5].

T. vulgaris L. is reported with an array of ethnobotanical applications due to its extensive pharmacological properties. The plant was chiefly used for the treatment of wounds, as it possesses healing and antiseptic properties [4, 5]. Usage of its aerial parts for fumigation, treating skin and respiratory diseases in ancient Europe deliberates on the anti-infective property of the plant [5]. Besides, *T. vulgaris* L. was used in monasteries in food preparations. These properties depict its efficacy as a culinary and medicinal plant as well. It is believed that Romans and Greek fumigated their surroundings by burning the entire plant [8]. Treatment of skin diseases like Black Death during the 1340s and several foodborne illnesses was also done using the *T. vulgaris* L. aqueous extract [5, 9, 10]. This is supported by modern studies, which have proved the

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antibacterial efficacy using both normal and MDR strains of virulent bacteria and fungi [11]. In the 1980s, *T. vulgaris* L. was recommended for treating respiratory disorders raised due to the inflammation of upper respiratory tract mucous membranes, including whooping cough, bronchitis, and catarrh [9]. However, application of thyme (*T. vulgaris* L.) and primrose (*Primula vulgaris*) extract has demonstrated beneficiary in clinical trials against bronchitis and other respiratory disease related symptoms [12]. Experts have also recommended the use of *T. vulgaris* L. in cases of bacterial and fungal infections. The plant is expected to render a possible inhibition of bacterial adsorption and biofilm matrix formation [13]. In addition, *T. vulgaris* L. was believed to possess antiseptic, astringent, carminative, tonic, and anthelminthic properties. The plant has gained much popularity due to the profound activity shown towards intestinal infections and infestations caused by ascarids, hookworms, fungi, yeast, and bacteria. It has also found its importance in dermatological issues like acne, oily skin, dermatitis, bug bites as well as sciatica and rheumatic aches. In support of this, modern pharmacological investigations have come up with several findings that satisfactorily decipher the antifungal nature of different extracts and compounds of the plant [14, 15, 16]. It is reported that, *T. vulgaris* L. relieves bites and stings, and the neurological issues associated with it. In aromatherapy, red *T. vulgaris* L. oil and white *T. vulgaris* L. oil are used to treat skin disorders as well as body pains [17, 18]. Modern anti-inflammatory investigations have revealed the efficacy of *T. vulgaris* L. in the amelioration of oxidative stress and cell mediated immune response [19, 20, 21].

These initial findings prevail the significant therapeutic potential of the plant with respect to pharmacological properties, which may be the results of its active components. In recent years, *T. vulgaris* L. has been the centre of research interest and colossal amount literature is already available. Although a few small reviews have been carried out on limited aspects of *T. vulgaris* L. [3, 22], no comprehensive review has been published till date including details of the potential therapeutic aspects. Therefore, we deliberate on summing up the present and up-to-date information with respect to chemical composition, ethnopharmacology, pharmacology, and toxicology of *T. vulgaris* L. We also aim to document a few uncommon pharmacological activities that can completely change the present perspectives on the plant.

2. Materials and methods

2.1. Databases, softwares, e-sources, and keywords search

Literature survey was conducted to gather all the essential information surrounding *T. vulgaris* L. using electronic databases including Google Scholar, PubMed, SpringerLink, Wiley-Blackwell, and Web of Science. An extensive number of studies published in peer reviewed journals like Food Chemistry, International Journal of Molecular Sciences, Fitoterapia, Journal of Science in Food and Agriculture, Parasitology, Toxicology research and many others were collected. E-books "Thyme: the genus *Thymus*" and "Medicinal spices and vegetables from Africa" were utilized as well. Authors searched for the data using keywords including "*Thymus vulgaris*," "*T. vulgaris*," "Ethnopharmacology," "Traditional uses," "Ethnobotany," "Chemical profiling," "Pharmacological properties," "Medicinal properties", which resulted in the gathering of much literature. A special search was conducted to get the ethnomedicinal details of the plant using words "root", "stem", and "leaves". Plant.org was used for the correct names of the plants used in the article. Also, chemical structures and IUPAC names were added using PubChem. The systematic arrangement of studies and their management till the end of the drafting was completed using the software Mendeley.

2.2. Literature management

Authors searched different databases using keywords to end up with a mammoth literature that included research articles, review articles, book chapters, and books. The literature was divided into different sections based on the title and abstract available along with the removal of non-relevant information and duplicates. Later, a second screening was carried out, where studies with unique methodology covering maximum research aspects were retained. Further, a few of the review articles with limited amount of information were removed. During the drafting of the review, a few research articles were removed to retain the articles with unique methodology and significant results. However, it was inevitable to retain a few of the articles despite their insignificant results and older methodology, due to lack of relevant and advanced information. From 2791 identified studies, a total of 118 studies were retained on completion of the different levels of screening. The outline was planned to meet up the PRISMA regulations (Figure 1). The article was primarily divided into different sections based on the previous work of authors on phytochemistry and pharmacological reviews. Further, a few additional changes were brought according to the amount and type of literature available.

3. Botanical background

3.1. Taxonomy

Being a member of the *Lamiaceae* family, the genus *Thymus* comprises a total of 928 species [23]. The most related genera are *Origanum*, *Satureja*, *Micromeria* and *Thymbra* [24]. The classification of *Thymus* species attributes to the chromosomal information, which is an important key for taxonomy. It is difficult to identify the chromosomes of *Thymus* species due to their small size and similar morphology [25].

3.2. Botanical description

T. vulgaris L. is an aromatic, perennial, straight growing plant which measures up 10–30 cm in height with woody base [24, 26]. Leaves are small, opposite, greyish green coloured, oblong–lanceolate to linear, and are gland-dotted. They are measured up to 5–10 mm long and 0.8–2.5 mm wide with recurved margins. Flowers are light violet in colour, two-lipped, and possess a hairy glandular calyx. They measure up to 5 mm long with leaf-like bracts in loose whorls arranged in axillary clusters on the branchlets or in terminal oval or rounded heads [26]. Figure 2 details the different parts of *T. vulgaris* L. However, the morphological characters may vary according to environmental conditions. *T. vulgaris* L. grows well in arid, temperate, and unshaded areas. *T. vulgaris* L. grows well in hot, arid conditions with well-drained soil, and is usually planted in the spring. The plant can be propagated using seed, cuttings, or by dividing rooted sections. The plant also takes up deep freezes and can be found on mountain highlands [4].

3.3. Variations

T. vulgaris L. shows aneuploidy where the number of chromosomes varies in different plants of the same species. Among these, $2n = 28, 30, 56$ and 60 are said to be the most frequent numbers for other plants belonging to the genus *Thymus*. Aneuploidy has played an important role during evolution and is responsible for varying numbers. This is true for *T. vulgaris* L. with $2n = 28, 58$ [24]. Thus, it becomes evident that the plant has a diverse genetic constitution. This may also contribute to its morphological variation. However, there are not available data on the restriction of these genetically diverse species to a specific region or country. Several sub-species are present that further add to the complexities of genetic research. In addition, several chemotypes or chemovars of *T. vulgaris* L. exist. As the phytochemical profile of *T. vulgaris* L. depicts the presence of extensive amount of essential oil components, chemotypes vary based on the composition of the same [27]. There are as many as 13 different chemotypes have been identified based on the predominance of monoterpenes in the essential oil. Recently, a study identified 6 different chemotypes namely linalool, borneol, geraniol, sabinene hydrate, thymol, and carvacrol [10]. Pharmacological effects of

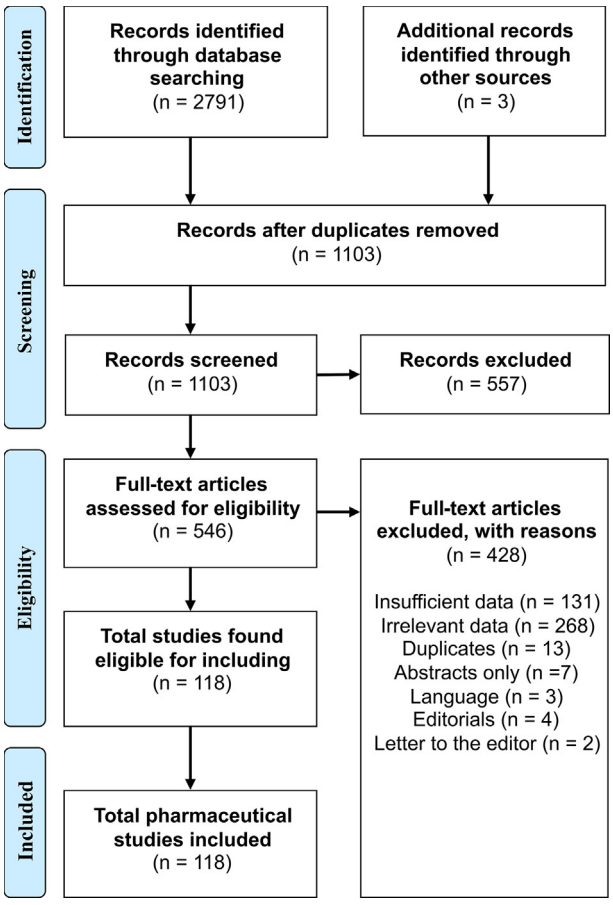

Figure 1. PRISMA outline followed for literature search.

different chemotypes vary due to their diverse essential oil composition [28, 29]. More research needs to be conducted in terms of chemotypes to identify the potent chemicals that can be used to target diseases at a molecular level. Based on the chemical profile and mode of employment of these chemotypes against specific diseases, significant results can be expected in phytotherapy.

3.4. Distribution

Native to Southern Europe, *T. vulgaris* L. has a worldwide distribution. It is indigenous to the Mediterranean region and other neighbouring countries. It is also found in Northern Africa including Egypt and Saharan countries like Morocco, Algeria, Tunisia, and Libya [9]. Also, the plant is cultivated in Nigeria, Cameroon, and South Africa. It is also cultivated in European countries, such as Spain, France, Bulgaria, Italy. Along the Mediterranean coastal region, it can be found growing up to 800 m from sea level. The plant can be cultivated by vegetative propagation, using seeds, cuttings, or divided root sections [2].

4. Ethnopharmacology

Thymus species have been used since ancient times for the treatment of different health aberrations. The plant possesses a variety of the medicinal properties that positively affect human health (Table 1). Authors were not able to find most of the specific parts of the plant used in traditional medicinal practices. Even though search was conducted using the words "root," "stem," or "leaves", there was no available records for specific parts of the plant used for traditional medicine. Few available studies were able to depict the details limited to "aerial parts" of the plant, which includes stem, leaves, flowers, and buds. Also, these studies were not able to reveal the accurate use of the plant. Mechanisms of ethnopharmacological uses were not available. The information was limited to "whole plant" or "thyme water". Investigations need to be done in this regard to decipher the unknown medicinal properties, which may benefit the pharmacological studies.

4.1. Treatment for poisoning

The aqueous extract of the plant is reported to possess the properties of an antidote. It is believed that Romans used to consume the plant extract before or during meal to protect themselves from getting poisoned. Bathing in warm water dosed with *T. vulgaris* L. could stop the effects of poison, making it a favourite herb for the emperors [8]. These examples indicate the effectiveness of *T. vulgaris* L. as a natural antidote. In support of this, the present-day studies show presence of compounds like thymol and carvacrol, which can serve as a profound antidote [31, 32].

4.2. Disinfection and wound healing

T. vulgaris L.is reported to be used as a disinfectant, where dried plant bundles were burned to purify the surroundings. Romans and Greeks evoked a spirit of courage by burning these bundles to purify their homes and temples [8]. This reminds us of the modern-day fumigation, where the disinfection is done with various antimicrobial substances. However, studies have proven the presence of γ-terpinene and p-cymene, which are the biochemical precursors of thymol and carvacrol, to be responsible for the observed antimicrobial properties [16, 33]. Nurses in the 19th century used to apply bandages soaked in thyme water, as this plant was believed to be a natural healer and an antiseptic. Also, the examination of the aqueous extract of *T. vulgaris* L. has been proved to improve immunomodulatory functions [34].

4.3. Treatment of skin diseases

T. vulgaris L. was also extensively used against plague in the late 1340s, an era that was known as the age of Black Death. *T. vulgaris* L. was directly applied on plague-blistered skin [5]. The beneficiary effects of such an application was later identified to be owing to the presence of a chemical compound known as thymol, which is widely used in hand sanitizers, mouthwashes, and acne medication in the present day. Also, several studies have proved the use of *T. vulgaris* L. for the treatment of skin problems like dermatitis [9]. These effects are observed due to the presence of volatile oils, which principally comprises linalool, α-pinene along with thymol. Other compounds like mono and sesquiterpenes (β-caryophyllene, germacrene-D and nerolidol) also reported to play an important role [33].

4.4. Treatment of foodborne illnesses

Apart from medicinal purpose, *T. vulgaris* L. has found its importance as a cooking herb alongside rosemary and sage in Europe. Monastries that served food, also used this plant as a food additive to wade away the microbial contamination as well as a part of the medicinal formulations. Food products like soups, roasts, and breads contained the plant formulation [10]. However, most of the studies have reported the antimicrobial activity of *T. vulgaris* L. extracts (ethanol and water) as well as its use in the form of essential oil against foodborne pathogens. In addition to thymol and carvacrol, phenolic compounds in the extracts are attributed for this property [35, 36].

4.5. Treatment of respiratory diseases

The whole plant extract was reported to be used against several respiratory disorders. It was believed to be an effective remedy for

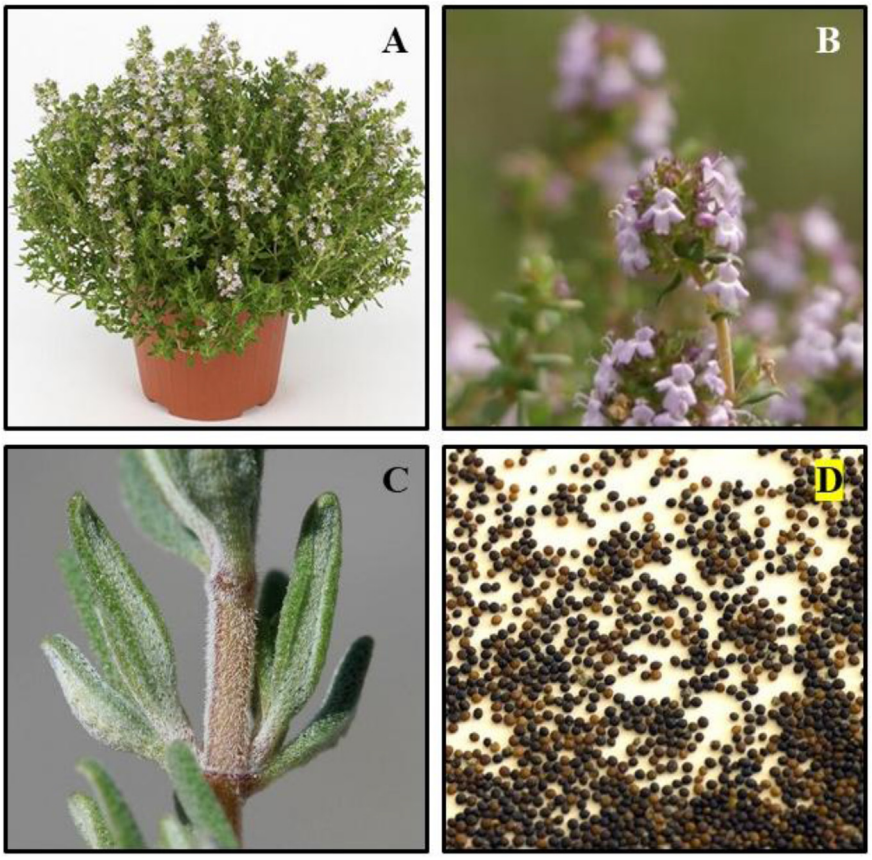

Figure 2. A) Plant B) flowers C) leaves D) seeds of *Thymus vulgaris* L.

Table 1. Summary of the ethnomedicinal uses of *Thymus vulgaris* L.

Ethnomedicinal Use	Part of the Plant	Palnt Material	Mode of Application	References
Treatment of poisoning	Aerial parts/stem/leaves	Dried whole plant/water extract	Oral, Topical	[8]
Disinfection and wound healing	Aerial parts/stem/leaves	Dried whole plant/vapours, water extract	Topical	[8]
Plague-blistered skin, Acne, oily skin, dermatitis	Aerial parts/stem/leaves	Water extract, volatile oil	Topical	[9, 18]
Treatment of foodborne illnesses	Aerial parts/stem/leaves	Dried whole plant/Water extract	Oral	[10]
Asthma, bronchitis, whooping cough, pharyngitis	Aerial parts/stem/Leaves	Water extract	Oral	[37]
Cough, cold and sore throat	Aerial parts/stem/Leaves/Flower shoots	Water extract	Oral	[37]
Intestinal worms	Aerial parts/stem/Leaves	Dried whole plant/water extract	Oral	[37]
Rheumatic aches, body pain and sciatica	Aerial parts/stem/Leaves	Volatile oil	Topical	[17]
Emphysema, sedative, anthelmintic, antifungal, antispasmodic, diuretic	Not defined	Not defined	Not defined	[41]
Skin infections, high blood pressure, heart problems, fluid retention, cystitis, digestive system disorders, rheumatism and arthritis	Flower shoots	Not defined	Not defined	[38]

bronchitis, asthma, whooping cough, and pharyngitis. *T. vulgaris* L. tea was extensively used to treat cough and cold. They were used to treat sore throat [37]. In addition, flowering shoots were used to treat cold, chest infections and sore throat [38]. Recently, the phytochemical analysis of the plant extracts revealed the presence of carvacrol and γ-terpinene that possess antiviral and anti-inflammatory activities, which could be the reason for the observed effects during its traditional use [32, 39].

4.6. Other traditional uses

T. vulgaris L. has been an important medication in several other health maladies. The plant possesses the ability to cure gastrointestinal aberrations, as the extract was given to treat worms in children [37]. The modern-day studies have reported the anti-parasitic/anti-helminthic role of *T. vulgaris* L., which is attributed to the presence of monoterpenes and phenolic compounds [40]. Also, the topical use of *T. vulgaris* L. oil reduced rheumatic aches and sciatica, where the aromatherapy method used the oil to treat such body pains [17]. This property of reducing pain is mainly attributed to thymol, which possesses the greatest anti-inflammatory potential among all the reported constituents. Flower shoots were used to treat mouth and skin infections, high blood pressure, heart problems, fluid retention, cystitis, digestive system disorders, rheumatism, and arthritis [38]. However, no specific phytochemicals have been attributed to these medicinal properties. In addition, reports have revealed the anthelminthic, antifungal, antispasmodic, diuretic [41], intestinal anti-inflammatory, buccal antiseptic, ocular antiseptic,

internal antiseptic, laxative, antiodontalgic, anticatarrhal, and urinary antiseptic [38] properties of the plant, yet without attributing the specific protogonistic phytochemicals.

5. Phytochemistry

T. vulgaris L. primarily consists of a myriad of chemical compounds categorised as phenolic compounds, terpenoids, flavonoids, steroids, alkaloids, tannins, and saponins. Most of them are volatile compounds extracted from plant oil. The studies have assessed the phytochemical composition mostly using gas chromatography-mass spectroscopy (GC-MS) and high-performance liquid chromatography/thin layer chromatography (HPLC/HPTLC) techniques [42, 43, 44]. The revelations depict the prevalence of essential oil components over the other metabolites.

Further, only a few studies could decipher the presence of steroids, alkaloids, tannins, and saponins, yet no information is available on individual compounds belonging into these categories [45, 46]. Apart from the plant oil characterisation, ethanolic and aqueous extracts need to be studied in detail to reveal information on the presence of various other important components. Several phytochemicals identified from this plant are of substantial pharmacological significance (Table 2). Though chemicals are present in *T. vulgaris* L., their isolation and identification has not been performed. Researchers have reported the activities of these phytochemicals from independent origin.

Table 2. Summary of the individually reported phytochemicals and their pharmacological significance present in *Thymus vulgaris* Linn.

Class	Compound Name	IUPAC Name	Pharmacological Activity	Reference
Phenolic compounds	Quinic acid	6-methoxyquinoline-4-carboxylic acid	Anti-cancer, immunomodulatory, anti-fungal, antioxidant, neuroprotective	[47, 83, 84]
	Rosmaric acid	(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl) prop-2-enoyl] oxypropanoic acid	Anti-alzheimer's, anti-cancer, antidiabetic, antimicrobial, cardioprotective, nephroprotective, anti-ageing, hepatoprotective, anti-inflammatory, anti-allergic, anti-depressant	[85, 86]
	Caffeic acid	(E)-3-(3,4-dihydroxyphenyl) prop-2-enoic acid	Antioxidant, antimicrobial, anticancer, adipogenetic, lipolytic, anti-alzheimer's, antiviral, antidiabetic, cardioprotective, hepatoprotective, anti-atherosclerotic	[87, 88, 89]
	p-coumaric acid	(E)-3-(4-hydroxyphenyl) prop-2-enoic acid	Immunomodulatory, anti-inflammatory, antioxidant, gastroprotective, antidiabetic, anti-hyperlipidemia, anti-tyrosinase, anticancer, hepatoprotective	[90, 91, 92]
	p-hydroxybenzoic acid	4-hydroxybenzoic acid	Anticancer, antimicrobial, antiviral	[93, 94, 95]
	Gentisic acid	2,5-dihydroxybenzoic acid	Anticancer, antioxidant, antimicrobial, cardioprotective, antimicrobial, anti-inflammatory, analgesic, nephroprotective, hepatoprotective, neuroprotective, muscle relaxant	[91,96,97]
	Syringic acid	4-hydroxy-3,5-dimethoxybenzoic acid	Anticancer, antioxidant, antidiabetic, anti-inflammatory, neuroprotective, antimicrobial, antiendotoxic, hepatoprotective, anti-osteoporotic	[98, 99]
	Ferulic acid	(E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid	Anticancer, antidiabetic, antioxidant, cardioprotective, neuroprotective, anti-alzheimer's,	[100, 101]
Terpenoids	Thymol	5-methyl-2-propan-2-ylphenol	Antibacterial, antifungal, antispasmodic, antitussive, anxiolytic, neuroprotective, antihypertensive, antioxidant, antihyperlipidemic, anti-inflammatory, immunomodulatory, anti-cancerous, analgesic, growth promoting	[41]
	Carvacrol	2-methyl-5-propan-2-ylphenol	Antimicrobial, antimutagenic, antitumor, analgesic, anti-inflammatory, antihepatotoxic, antiparasitic, antispasmodic, and hepatoprotective	[32]
	Geraniol	(2E)-3,7-dimethylocta-2,6-dien-1-ol	Anti-cancerous, anti-inflammatory, antioxidant, hepatoprotective, antimicrobial, cardioprotective, antidiabetic, neuroprotective	[102]
	Linalool	3,7-dimethylocta-1,6-dien-3-ol	Sedative, antiviral, anti-inflammatory, antioxidant, anti-nociceptive, analgesic, anesthetic, antimicrobial, anxiolytic, anti-hyperlipidemic, antinoceptive, antidepressive, neuroprotective	[41, 103]
	ρ-Cymene	1-methyl-4-propan-2-ylbenzene	Antimicrobial, antinociceptive and anti-inflammatory, antioxidant, anxiolytic, anticancer, vasorelaxant, immunomodulatory, antinoceptive	[104, 105, 106]
	γ-terpinene	1-methyl-4-propan-2-ylcyclohexa-1,4-diene	Antibacterial, antioxidant, anti-inflammatory, antinociceptive.	[107, 108, 109]
	Limonene	1-methyl-4-(1-methylethenyl)-cyclohexene	Antibacterial, antifungal, anti-inflammatory, antinociceptive, antioxidant,	[110, 111]
	β-Caryophyllene	(1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene	Antimicrobial, cardioprotective, hepatoprotective, gastroprotective, neuroprotective, nephroprotective, antioxidant, anti-inflammatory, immunomodulatory	[48]
	β-Pinene	6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane	Anti-cancerous, antimicrobial, antioxidant, anti-inflammatory, analgesic, cytogenetic, gastroprotective, anxiolytic, cytoprotective, anticonvulsant, neuroprotective	[49]
	α-Terpineol	2-(4-methylcyclohex-3-en-1-yl) propan-2-ol	Anti-cancerous, antioxidant, antinociceptive, anticonvoluscent, gastroprotective, cardioprotective, antihypertensive, sedative	[112]
Flavonoids	Apigenin	5,7-dihydroxy-2-(4-hydroxyphenyl) chromen-4-one	Antidiabetic, anti-cancerous, antidepressive, anti-insomnia, anti-amnesia, anti-alzheimer's, antiviral	[113]
	Luteolin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	Anti-alzheimer's, anti-cancerous	[114, 115]
	Cirsimaritin	5-hydroxy-2-(4-hydroxyphenyl)-6, 7-dimethoxychromen-4-one	Antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, neuroprotective, cardiovascular, hepatoprotective	[116]
	Xanthomicrol	5-hydroxy-2-(4-hydroxyphenyl)-6,7, 8-trimethoxychromen-4-one	Anti-inflammatory, anti-spasmodic, anti-platelet, anti-cancerous effects	[117, 118]

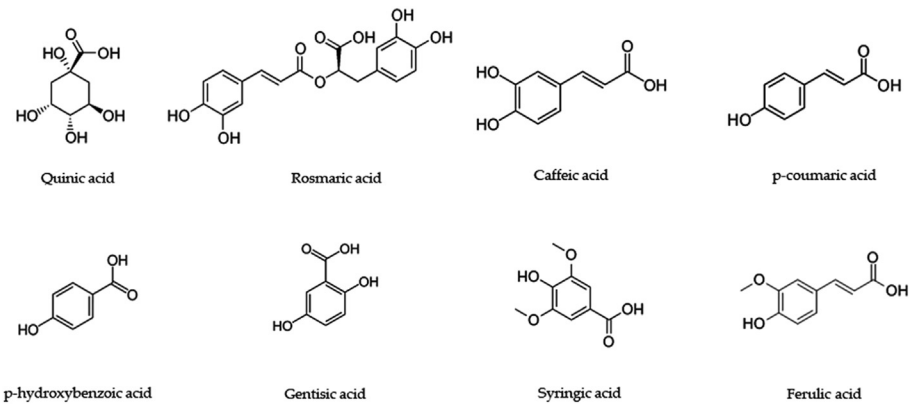

Figure 3. Phenolic compounds with pharmacological activity in *Thymus vulgaris* L.

5.1. Phenolic compounds

T. vulgaris L. is one of the principal sources of phenolic compounds. Heidari et al. (2018) identified new phenolic compounds using GC-MS technique, in which a few of the phenolic compounds were already reported. They include creosol 2-Methoxy-4-methylphenol), thiophenol (benzenethiol), quininic acid (6-methoxyquinoline-4-carboxylic acid), loliolide [(6S,7aR)-6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one], phenol 4-(3- hydroxy-1- propenyl-2- methoxy), and 3-Methoxy-5- methylphenol [47]. Also, previous studies have identified the presence of rosmaric acid, caffeic acid, p-coumaric acid, geranic acid, p-hydroxybenzoic acid, gentisic acid, syringic acid, and ferulic acid [4]. Among the various others reported, the highest number of compounds with pharmacological significance were the phenolic compounds (Figure 3).

5.2. Terpenoids

Characterization of the essential oil of *T. vulgaris* L. reveals a list of hydrocarbons, oxides, alcohols/esters, and aldehydes/ketones. Many of the studies have reported the uses of a variable number of compounds with different concentrations. Among all the reported volatile compounds, thymol, carvacrol, geraniol, linalool, α- and β-pinene, p-cymene, and γ-terpinene have been reported with major pharmacological activities [48, 49, 50]. In addition, hydrocarbons like 2,6-Octadienal, cis-Sabinene hydrate, germacrene D, limonene, β-ocimene, myrcene, β-caryophyllene, α-thujene, α-phellandrene and α-humulene are present. Oxides like 1,8-cineol, caryophyllene oxide; alcohol/esters including α-terpineol, borneol, 1-octen-3-ol, 3-octanol, p-cymen-8-ol, terpinen-4-ol, thymol methyl ether, carvacrol methyl ether; aldehydes/ketones like 3-octanone, camphor, thymoquinone, and geranial are present. The GC-MS analysis also showed the presence of esters including butanoic acid, 2-methyl-, methyl ester, bornyl acetate, and geranyl propanoate. The majority of these compounds need to be assessed individually to decipher their pharmacological potential [51]. However, some of the terpenoids have been reported with profound pharmacological significance (Figure 4).

5.3. Flavonoids

Flavones like 6-hydroxyluteolin, apigenin, luteolin, methyl-flavones including cirsimaritin or genkwanin, cirsilineol, 5-desmethylnobiletin, 8-methoxycirsilineol, 7-methoxyluteolin, gardenin B, salvigenin, thymonin, sideritoflavone, xanthomicrol and thymusin [4]. There have been no reports on pharmacological activities of these compounds except apigenin, luteolin, cirsimaritin, genkwanin, and xanthomicrol. Studies need to focus on the extraction and biosynthesis of individual compounds which can further become a basis for phytomedicines. Although only a few of the flavonoids are reported (Figure 5), they are found to exhibit profound pharmacological activities.

5.4. Steroids, tannins, alkaloids, saponins

A study that used HPTLC to assess the methanolic extract of *T. vulgaris* L. documented the presence of 9 alkaloids, 14 saponins, 12 steroids, and 8 tannins. The study revealed a good resolution with 10 bands of essential oils, along with bands corresponding to tannins (9.2%) and saponins (23.1%), where a remarkable antimicrobial activity is possessed

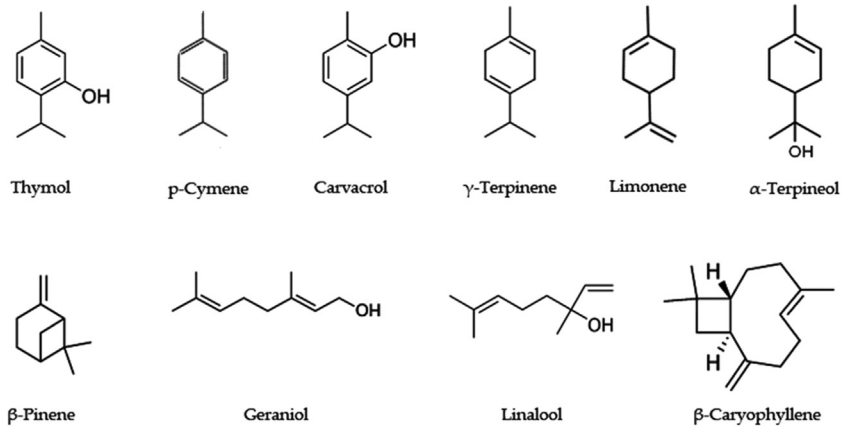

Figure 4. Terpenoids with pharmacological activity in *Thymus vulgaris* L.

by saponins [46]. However, it could only reveal the presence of a class of compounds but not decipher the presence and role of individual compounds. As most of the chemical components belong to essential oils and phenolic compounds, metabolites like steroids, tannins, alkaloids, and saponins have not been evaluated in detail. Therefore, it now becomes essential to focus on these compounds to reveal their biological significance.

5.5. Other phytochemical compounds

Banerjee et al. (2019) assessed polysaccharide content using aqueous extraction of the alcohol insoluble residue of *T. vulgaris* L. leaf and reported the presence of four types of polysaccharides. They include homogalacturonan, starch, cellulose and a unique type of polysaccharide known as rhamnogalacturonan I (RG-I). They also reported the association of RG-I with ester linked phenolic acids shows profound antioxidant activity [52]. However, much remains unknown about other biomolecules present in *T. vulgaris* L.. Identification of the other biomolecules like proteins and vitamins using the whole plant extracts could lead to the elucidation of nutritive profiling of the plant.

6. Pharmacology

6.1. Antibacterial activity

Bacteria obtained from the feces of the red deer (*Cervus elaphus*) including *Escherichia coli, Klebsiella pneumoniae, Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis* were found sensitive towards the ethanol extract of *T. vulgaris* L., where minimum inhibitory concentration (MIC) was observed as >6.25, 3.12,>6.25, 0.2, 0.39, 0.78 mm respectively at 100 µl concentration ($p < 0.05$). These bacteria showed mixed response towards antibiotics [53]. In another study, methanolic extract of *T. vulgaris* L. was found to be effective against methicillin-resistant *S. aureus* (MRSA), where bacteria isolated from the infected mice body parts were tested under *in vivo* conditions. It was found that for the bacteria isolated from throat and lungs, MIC was found to be 2.93 and 3.83 colony forming unit (CFU) (log_{10})/ml, respectively ($p < 0.05$) [54]. In case of *Salmonella typhirium*, thyme oil from *T. vulgaris* L. showed MIC of 25.5 mm at 100 µl concentration, in accordance with amoxicillin (23.0 mm) and cefotaxime (15.0 mm) [55]. Biofilm of *Salmonella enteritidis* (48 h) was inhibited by thyme oil at an MIC/MBC of 0.156/0.315 µl/ml, in accordance with that of the control. The oil also reduced the metabolic activity by 9.6–70.5%, where both biofilm and metabolic activity reductions were found to be significant ($p < 0.05$). The study also included the individual assessment of thymol and carvacrol, whose results were found to be strikingly similar in case of antibacterial assay [13].

Further, the essential oil from *T. vulgaris* L. was found to inhibit the growth of multi-drug resistant (MDR) variants of *S. aureus*. A significant inhibition was observed with a zone of 35–40 mm at 2.8–11.5 µg/ml for MDR variants, whereas cefotaxime showed MIC at 32 µg/ml concentration ($p < 0.05$) [11]. In addition, *Pseudomonas aeruginosa* swabs were taken from surgical wounds at the end of the hip implant surgery, where minimum bactericidal concentration (MBC) was found to be 8% at 100 µl concentration [56]. *T. vulgaris* L. essential oil or thyme oil was also found to be effective against porcine respiratory bacteria. *Actinobacillu spleuropneumoniae, Streptococcus suis, Actinobacillus suis, Haemophilus parasuis, Pasteurella multocida,* and *Bordetella bronchiseptica* were tested with thyme oil and considerable MIC values were obtained, ranging from 0.039% to 0.078% at dilutions 0.01–1.25% v/v ($p < 0.05$) [57].

These studies indicate the antibacterial potential of thyme oil and other extracts on bacteria isolated from various sources. A greater inhibitory activity was observed by the use of thyme oil against bacteria in comparison with that of the aqueous, ethanolic extracts, and even a few antibiotics [55]. Thyme oil is also capable of inhibiting biofilm formations, which are regarded to be highly infective [13]. Although both bacteriostatic and bactericidal values were discovered, dose-dependent activities need to be conducted using animal models and human cell lines. It is noteworthy that thyme oil has exhibited a significant bacteriostatic activity against MDR and MRSA strains [54, 56]. Though the oil serves as a better antibacterial agent owing to its facile extraction method and significant activity, studies need to focus on individual compounds to assess their effects either in single or in combination. Many of the studies have reported the synthesis as well as extraction of individual compounds present in thyme oil that are proved to have profound antibacterial effects [11, 13, 56, 57]. Further, mechanisms of the bacteriostatic and bactericidal functions of the fractions need to be elucidated with respect to physicochemical and physiological changes in bacterial cell.

6.2. Antioxidant activity

Antioxidant enzymes like catalase, glutathione, glutathione-S-transferase, and superoxide dismutase are primarily responsible for the reduction of free radicals in the body. Abdel-Gabbar et al. (2019) assessed the *in vivo* activity of these enzymes by the administration of *T. vulgaris* L. aqueous extract on the experimental rabbits, where the levels were found to be increased by 14.12%, 27.69%, 98.75% and 78.29%, respectively ($p < 0.05$) compared with that of the control (water). Total antioxidant capacity was increased at 100 mg/kg and 50 mg/kg concentration in rabbits, with no adverse effects on kidney and liver function parameters [58]. Similarly, the levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were also significantly increased (2 units/ml) upon the administration of 500 mg/kg of *T. vulgaris* L. aqueous extract for 14 days. In combination with paracetamol, (200 mg/kg), the enzyme levels increased by 15–20 units/ml, compared with that of the control ($p < 0.01$) [59]. In addition, the antioxidant potential of the aqueous extract was also assessed using conventional methods. The aqueous extract of *T. vulgaris* L. leaf and stem was analysed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) resulting in the free radical scavenging activity (92.0%) at a concentration of 1.5 mg/ml, in accordance with butylated hydroxyanisole (BHA-95.7%) and butylated hydroxytoluene (BHT-96.6%). The potential benefits observed in the study were attributed to the presence of polysaccharides like starch, homogalacturonan, rhamnogalacturonan I (RG-I) and cellulose in *T. vulgaris* L. leaves. The binding of RG-I with bovine serum albumin (BSA) indicated the formation of water-soluble complexes that further induces antioxidant activity [52].

In case of thyme oil, DPPH, Ferric reducing antioxidant power (FRAP), Ferrous ion-chelating ability assay (FIC), and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activities were reported with the IC_{50} values 12.69, 13.29, and 6.46 mg/ml, respectively ($p < 0.05$) [60]. The unused plant material obtained after extracting the oil using steam distillation process was also found to be the reservoir of phenolic compounds. El-Guendouz et al. (2019)

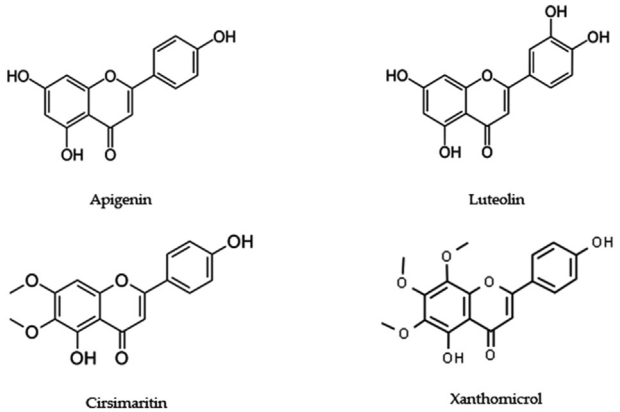

Figure 5. Flavonoids with pharmacological activity in *Thymus vulgaris* L.

demonstrated the ability of the ethanolic extract obtained using the oil extraction by-product of *T. vulgaris* L. in preventing the formation of primary and secondary lipid oxidation products from oil in water (O/W) emulsions. Assessment of the antioxidant activity through DPPH showed an IC_{50} of 93 μg/ml, similar to that of BHT (89 μg/ml). But these values were found to be of lower activity compared with that of the pure aqueous and ethanol extracts [61].

The antioxidant potential of the oil is proved to be higher than those of the aqueous and ethanolic extracts [60, 61]. However, novel applications like silver and zinc nanoparticles could be used in place of pure extracts, as they possess lower amount of cytotoxicity. Usage of cell lines and animal models could provide a better insight to the studies, revealing the biochemical changes that could occur before and after the administration of the *T. vulgaris* L. fractions. It is noteworthy that the antioxidant enzymes have been focused rather than the conventional assays like DPPH, ABTS, and FRAP [58, 59]. However, it is advisable that these enzymatic analyses could be carried out using human cell lines with correlation in terms of its toxicity based on dose-dependent studies. In addition, studies have proved that different *T. vulgaris* L. extracts could alone be able to reduce the oxidative stress levels using the conventional antioxidant assays. But there are no available controls to compare its efficacy [58, 59]. In addition, the treatment with aqueous *T. vulgaris* L. extract alone has proved its potential to prevent or reverse the drug-induced toxic biochemical changes to normal via its antioxidant activity [58]. As a key finding, studies have revealed that an array of bioactive compounds showing antioxidant activities are also present in leaves and other parts of the plant, other than the oil alone [52].

6.3. Antifungal activity

Thyme essential oil was found to be effective against fungal species including *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Phytophthora parasitica*, *Pythium aphanidermatum*, *Fusarium oxysporum*, *Alternaria brassicae*, *Trichoderma aggressivum* f.sp. *europaeum*, and *Cladobotryum mycophilum*. The sensitivity of these fungi were assessed with the radial growth inhibition assay, which revealed an inhibitory value ranging from 13.9 to 41.4 mm at 5% concentration and ED_{50} values ranging from 9.3 to 18.0% for all the species tested ($p < 0.05$) [14]. Further, a group of 183 clinical isolates of *Candida albicans* and 76 isolates of *C. glabrata* species were tested for sensitivity towards *T. vulgaris* L. extract. Both MIC and minimum fungicidal concentration (MFC) were found to be in the range of 0.04–22.9 mg/ml for all the isolates. In the time kill assay, thyme oil reduced the fungal growth in the initial hours (4–8). Interestingly, thyme oil inhibited the fungal growth supplemented with sorbitol, an osmoprotectant with a much lower MIC (0.08 mg/ml) ($p < 0.05$) [15]. In addition, an *in vivo* assessment using thymol on *C. albicans* in a *Caenorhabditis elegans* nematode model resulted in the complete inhibition of the fungus at 64 mg/l and 128 mg/ml, better than that of the standard antibiotics used ($p < 0.05$). Thymol is also reported to enhance the expressions of pmk-1and sec-1 genes, thereby resulting in the enhancement of p38 MAPK signalling pathway, which exhibits signalling events to combat *C. albicans* [62].

Scalas et al. (2018) assessed the antifungal activity against *Cryptococcus neoformans* where MIC and MFC were found to be in the range of 56–1.12 mg/ml, in accordance with the standards fluconazole (FLC), itraconazole (ITC), and voriconazole (VRC). But individual antifungal assay showed profound results with thymol (0.02–0.08 mg/ml) ($p < 0.05$) [63]. Thyme oil in vapor and liquid phase was also found to be effective against fungi, where a total reduction in mycelial growth was detected at the concentrations of 20 and 400 μg/ml, respectively for *Aspergillus flavus*. Also, treatment with 10 μg/ml of thyme oil reduced production of aflatoxin by 97.0 and 56.4% through vapour and liquid phases, respectively ($p < 0.05$). In addition to this, the study also reported the downregulation of a few genes related to fungal development including brlA, abaA, and wetA and genes related to aflatoxin biosynthesis -aflR, aflD, and aflK [16].

It is noteworthy that a majority of the studies have performed both fungistatic and fungicidal assessments which determine the complete inhibition of fungi. A few of the studies have performed the time kill assay and *in vivo* inhibition assays to effectively determine the antifungal activity [15, 62]. The literature search was not able to find the antifungal studies completed using water and alcohol extracts. Also, there is a lack in antifungal studies performed, using the extracts and compounds on MDR species. The fungal resistance needs to be dealt with using variable number of doses of the extracts along with concerns regarding its cytotoxicity on the living cells. Furthermore, there is a lack of information on the fungicidal and fungistatic mechanism of the plant extracts and essential oil [15, 16, 62]. Owing to a lack of studies on host-pathogen interaction, advanced studies to decipher this mechanism should be carried out in order to identify the optimal dose. These studies must evaluate the cytotoxic effects as well as cell survivability so that clinical trials using plant fractions could be performed.

6.4. Anti-inflammatory activity

Though nitric oxide (NO) radicals act as intracellular messengers under normal conditions, their elevated numbers can bring up the cytotoxicity and inflammation issues. A study has evaluated the anti-inflammatory potential of *T. vulgaris* L. aqueous extract. Significant scavenging of NO radicals with 80.3% of the activity at 16 μg/ml concentration was observed in accordance with the values obtained using dexamethasone ($p < 0.05$) in murine macrophage cell line J774A.1 [19]. In case of inflammation, immune cells generally express the genes encoding for 5-lipoxygenease (5-LOX) production, which in turn activates the synthesis of leukotrienes. Tsai et al. (2011) targeted the inhibition of 5-LOX, showing an inhibition at 0.005 μg/ml (IC_{50}) of thyme oil concentration. This was more effective than α-bisabolol (0.049 μg/ml). They also assessed the effect of essential oil on lipopolysaccharide (LPS)-induced TNF-α, interleukins IL-1β, and IL-8 secretions using THP-1 cells at 0.01 μg/ml [20].

In an *in vivo* study, Abdelli et al. (2017) analysed thyme oil in a dose dependent manner (100, 200 and 400 mg/kg) against mice with carrageenan-induced paw edema to determine the anti-inflammatory activity. Paw thickness was found reducing at a dose of 400 mg/kg. Results were in accordance with both the controls Tween 80 and diclofenac ($p < 0.001$). The study also determined the toxic level of thyme oil (4500 mg/kg), where sedation was observed at 5000 mg/kg [43]. Similarly, in the carrageenan-induced pleurisy model thyme oil at 250, 500 and 750 mg/kg reduced inflammatory exudates as well as migrated leucocytes in ear edema. Individual assessments revealed that thymol (34.2%) and carvacrol (47.3%) are attributable for the anti-inflammatory activity ($p < 0.05$). This study also reported the toxicity level of thyme oil as 4000 mg/kg, which stands in accordance with the findings of Abdelli et al. (2017) [64].

It was reported that anti-inflammatory response can be effectively generated by both extracts and essential oil from *T. vulgaris* L [19, 20, 43, 62]. It is a laudable approach by the researchers to assess the levels of inflammatory cytokines and tumour necrosis factor (TNF) [20]. A study was even able to determine the toxicity level of the thyme oil as 4500 mg/kg in mice models [43]. However, a few notable parameters like cyclooxygenase-2 (COX-2), cholestasis, vascular lesions, c-reactive protein (CRP) in chronic hepatitis, hepatic fibrosis, drug-induced autoimmunity could be assessed, which can support the antioxidant potential. In addition, autoimmune models like rheumatoid arthritis could be focused to assess the effect of *T. vulgaris* L. fractions.

Table 3. Summary of the pharmacological properties of *Thymus vulgaris* L.

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
Antibacterial activity	*in vitro*	*Escherichia coli, Klebsiella pneumoniae, Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis*	Aerial parts	Ethanol	100 μl of 6.25–0.025% serial dilutions	Solvent ethanol	Not defined	MIC was observed as >6.25, 3.12,>6.25, 0.2, 0.39, 0.78 mm respectively at 100 μl concentration ($p < 0.05$). These bacteria showed mixed response towards antibiotics.	[53]
	in vivo	Methicillin-resistant *Staphylococcus aureus* (MRSA) in mice	Not defined	Methanol	200 mg/ml per kg of body weight	Positive control-infected, negative control-normal mice, Antibiotics	Not defined	For the bacteria isolated from throat and lungs, MIC was found to be 2.93 and 3.83 CFU (log_{10})/ml, respectively ($p < 0.05$).	[54]
	in vitro	*Salmonella typhirium*	Aerial parts	Essential oil	100 μl of 1/20–1/200 v/v serial dilutions	Amoxicillin, cefotaxime	Not defined	MIC of 25.5 mm at 100 μl concentration, in accordance with amoxicillin (23.0 mm) and cefotaxime (15.0 mm) ($p < 0.05$).	[55]
	in vitro	*Salmonella* Enteritidis Biofilm	Dried plant	Essential oil	5–0.0024 μl/ml.	Growth control (broth + microbe), negative control (broth + propylene glycol + microbe), sterility control (broth + test oil), positive control (broth + streptomycin + microbe)	Possible inhibition of bacterial adsorption and biofilm matrix formation	Biofilm inhibition at MIC/MBC 0.156/0.315 μl/ml by oil, thymol, and carvacrol. Oil reduced the metabolic activity by 9.6–70.5%, ($p < 0.05$).	[13]
	in vitro	*Staphylococcus aureus* (MDR)	Not defined	Essential oil	10 μl of 2.87–11.5 μg/ml	Cefotaxime	Not defined	A significant inhibition with 35–40 mm inhibition zone at 2.8–11.5 at μg/ml was observed for MDR variants, whereas cefotaxime showed MIC at 32 μg/mL concentration ($p < 0.05$)	[11]
	in vitro	*Pseudomonas aeruginosa*	Leaves and branches	Essential oil	100 μl oil of different concentrations	Not defined	Not defined	Minimum bactericidal concentration (MBC) was found to be 8% at 100 μl concentration	[56]
	in vitro	*Actinobacillus pleuropneumoniae, Streptococcus suis, Actinobacillussuis, Haemophilus parasuis, Pasteurella multocida, and Bordetella bronchiseptica*	Not defined	Essential oil	100 μl of 1.25 to 0.01% (v/v)	Media + microbes + PBS	Not defined	MIC values ranging from 0.039% to 0.078% at dilutions 0.01–1.25% v/v ($p < 0.05$)	[57]
Antioxidant activity	*in vivo*	Antioxidant enzyme levels in rabbits	Not defined	Aqueous extract	50 mg/kg of body weight	Water	Not defined	Levels of antioxidant enzymes catalase, glutathione, glutathione-S-transferase, and	[58]

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Table 3 (*continued*)

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
								superoxide dismutase increased by 14.12%, 27.69%, 98.75% and 78.29%, respectively ($p < 0.05$)	
	in vivo	Antioxidant enzyme levels in rats	Dried leaves	Aqueous extract	500 mg/kg body weight	Paracetamol (200 mg/kg)	Not defined	Alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase content increased by 2 units/mL. In combination with paracetamol, the enzyme levels increased by 15–20 units/ml ($p < 0.01$)	[59]
	in vitro	Radical scavenging activity using DPPH	Leaf and stem	Aqueous extract	0.0125–3.0 mg/ml	Not defined	Polysaccharide biding with BSA brings out the radical scavenging	Radical free scavenging activity of 92.0% at the concentration of 1.5 mg/ mL, in accordance with butylated hydroxyanisole (BHA-95.7%) and butylated hydroxytoluene (BHT-96.6%).	[52]
	in vitro	FRAP, ABTS, and FIC	Not defined	Essential oil	0.23–30 mg/ml	Not defined	Not defined	FRAP, FIC, ABTS assays showed IC_{50} values 12.69, 13.29, and 6.46 mg/ml respectively ($p < 0.05$)	[60]
	in vitro	Primary and secondary lipid oxidation products in oil in water (O/W) emulsions through DPPH	Dry waste plant	Ethanolic extract	100 µl of different concentrations	Not defined	Not defined	IC_{50} of 93 µg/ml compared to BHT (89 µg/ mL) ($p > 0.05$)	[61]
Antifungal activity	*in vitro*	*Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Phytophthora parasitica*, *Pythium aphanidermatum*, *Fusarium oxysporum*, *Alternaria brassicae*, *Trichoderma aggressivum*f.sp. *europaeum*, *Cladobotryum mycophilum*	Not defined	Essential oil	5,10,15,20,30% (v/v)	Media + Tween 20	Not defined	Mycelial growth inhibition values were found to be ranging between 13.9 to 41.4 mm at 5% concentration with ED_{50} values ranging from 9.3-18.0% for all the species ($p < 0.05$)	[14]
	in vitro	Clinical isolates of *Candida albicans* and *C. glabrata* species	Not defined	Essential oil	0.005–2.5% (v/v)	Amphotericin B	Possible ergosterol binding	MIC and MFC were in the range of 0.04–22.9 mg/ ml for all the isolates. Thyme oil reduced the fungal growth in the initial hours (4–8). It inhibited the growth with sorbitol at lower MIC (0.08 mg/ml) ($p < 0.05$)	[15]

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Table 3 (*continued*)

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
	in vivo	*C. albicans* in a *Caenorhabditis elegans* nematode model	Not defined	Thymol	32, 64, and 128 mg/l	Kanamycin (45 μg/ml), ampicillin (100 μg/mL), and streptomycin (100 μg/ml)	Enhancing pmk-1and sec-1 gene expressions, which in turn enhance p38 MAPK signalling pathway	Complete inhibition of fungi and biofilm at 64 mg/l and 128 mg/ml, compared to control used ($p < 0.05$). Growth reduction at 12 h, compared to control (36 h). Thymol enhances the expressions of pmk-1and sec-1 genes, in turn p38 MAPK signalling pathway	[62]
	in vitro	*Cryptococcus neoformans*	Not defined	Essential oil	0.07–10 mg/ml	FLC (0.06–128 μg/mL), ITC (0.0078–2 μg/ml), VRC (0.0078–32 μg/mL)	Possible membrane deterioration by thymol, Possible ergosterol binding	MIC and MFC were found to be in 0.56–1.12 mg/ml in accordance with controls. Thymol showed better activity 0.02–0.08 mg/ml ($p < 0.05$)	[63]
	in vitro	*Aspergillus flavus*	Not defined	Essential oil vapour and liquid phases	0, 1, 5, 10, and 20 μg/ml	Aflatoxin B	Downregulating of fungal development genes brlA, abaA, wetA and aflatoxin biosynthesis genes aflR, aflD, and aflK	Vapor and liquid phases reduced growth at 20 and 400 μg/ml, respectively. Thyme oil 10 μg/mL of reduced production of afltoxin by 97.0 and 56.4% through vapour and liquid phases, respectively. ($p < 0.05$).	[16]
Anti-inflammatory activity	*in vitro*	NO radical scavenging in murine macrophage cell line J774A.1	Flowering tops	Aqueous extract	8.5, 16, 50.4, 84 μg/ml	Dexamethasone	Possible cellular mechanisms of suppression of iNOS induction by flavonoids	Significant scavenging of NO radicals with 80.3% of the activity at 16 μg/ml concentration was observed in accordance with control ($p < 0.05$)	[19]
	in vitro	5-lipoxygenease (5-LOX) production, lipopolysaccharide (LPS) induced TNF-α, IL-1β, and IL-8 secretions using THP-1 cells	Dried plant	Essential oil	30 μl of different concentrations	α-bisabolol	Not defined	5-LOX got inhibited at 0.005 μg/ml (IC_{50}) of thyme oil, compared to α-bisabolol (0.049 μg/mL). TNF-α, IL-1β, and IL-8 got inhibited at 0.01 μg/ml.	[20]
	in vivo	Mice with carrageenan-induced paw edema	Aerial parts and dried leaves	Essential oil	100, 200 and 400 mg/kg	Tween 80 and diclofenac	Not defined	Paw thickness was found reducing at a dose of 400 mg/kg. Results were in accordance with both the controls Tween 80 and diclofenac ($p < 0.001$). Toxic level of thyme oil was found (4500 mg/kg), where sedation was observed at 5000 mg/kg.	[43]
	in vivo	Mice with carrageenan-induced pleurisy	Leaves	Essential oil	250, 500 and 750 mg/kg	Croton oil	Carvacrol may act by inhibiting cytokines and leukotrienes, and these mediators are likely not	All the concentrations reduced inflammatory exudates as well as migrated leucocytes in	[64]

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Table 3 (*continued*)

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
							involved in the mechanism of action of thymol	ear edema. Individual assessment showed thymol (34.2%) and carvacrol (47.3%) are attributable for the anti-inflammatory activity ($p < 0.05$)	
Anti-cancerous activity	*in vitro*	MCF7 (breast adenocarcinoma), HCT15 (colon carcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and NCI-H460 (non-small cell lung cancer) cell lines	Dried aerial parts	Essential oil	10–100 μg/ml	Ellipticine (0.24–65.2 μg/ml)	Possible involvement of thymol in the stimulation of active proliferation of pulp fibroblasts	*T. vulgaris* L. oil showed inhibition of growth at 76.02–180.40 μg/ml concentration (GI_{50}). It did not show any effect on non-tumour liver PLP cells, even at a high concentration of 400 μg/ml ($p < 0.05$)	[65]
	in vitro	THP-1 leukemia cell line	Not defined	Essential oil	10–500 μg/ml	DMSO	Not defined	At a concentration of 100 μg/ml and >200 μg/ml, thyme oil prevented the proliferation of THP-1 leukemia cells	[66]
	in vitro	H460 lung cancer cell line	Not defined	Hydroalcoholic extract	0.04–0.6%	Glyceraldehyde 3-phosphate dehydrogenase	Possible interference in pro-inflammatory cytokines	H460 lung cancer cell line was found to be sensitive at 0.11% of hydroalcoholic extract ($p < 0.05$) and downregulated NF-κB p65 and NF-κB p52 proteins along with the reduction of IL-1β and IL-8 gene expression in LPS model	[21]
	in vivo	Mammary carcinoma rat and 4T1 mouse models	Dried plant	Thyme powder	50 mg/kg body weight	Untreated models	Possible interference with pro-inflammatory cytokines, Possible upregulation of caspase genes at epigenetic level	Thyme powder reduced the volume of 4T1 tumours by 85% at 1% concentration. In rat model, the same concentration decreased the tumour frequency by 53% ($p < 0.05$). Upregulation of caspase-2 and caspase-3 enzymes, along with bcl-2 and Bax proteins	[67]
	in vitro	HL-60 acute promyelotic leukemia cell line, human peripheral blood mononuclear cell (PBMC)	Not defined	Thymol	5, 25, 50, 75 and 100 μM for 24 h	Camptothecin (5 μM)	Apoptosis induced by thymol in HL-60 cells was associated with ROS production, increase in mitochondrial H_2O_2 production, decrease in Bcl-2 protein, increase in Bax protein levels, enhancing apoptosis inducing factor (AIF) in	Thymol showed no cytotoxic effect on human peripheral blood mononuclear cell (PBMC) at 5 and 25μM concentrations. However, extensive cytotoxicity was observed at >50 μM, after 24 h	[68]

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Table 3 (*continued*)

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
							mitochondria and caspase activation		
	in vitro	Synthesized silver nanoparticles against T47D human breast cancer cells	Dried leaves	Silver nanoparticles and ethanol extract	12.5–200 μg/ml	Untreated cells	Nanoparticles could trigger translocation of phosphatidylserine (PS) from the inner membrane indicating apoptosis pathway rather than necrosis	T47D cells showed high sensitivity towards nanoparticles (90%) compared to the extract (75%). T47D cells treated with nanoparticles showed 18.40% early and 0.69% late apoptosis with varying IC_{50} concentrations (12.5–100 μg/mL). Same was observed in case of plant extract, where 15.67% early and 1.70% late apoptosis was found ($p < 0.05$)	[47]
Antiviral activity	*in vitro*	Influenza virus	Not defined	Essential oil vapour and liquid phases	3.12–100 μl/ml	Canova oil	Possible interaction with hemagglutinin (HA)	Liquid phase at 3.1 μl/ml concentration completely inhibited the viral growth, which was better than that of control used (canola oil). Significant inhibition of HA was observed. Also, 50% of the culture was reduced depicted as TC_{50} 14.34 μl/ml ($p < 0.05$)	[33]
	in vitro	Herpes simplex virus (HSV) on RC-37 (African green monkey kidney cells)	Not defined	Essential oil	10–750 μg/ml	Untreated cells	Not defined	Cytotoxicity ranged between 20 μg/ml for citral and 1250 μg/ml for 1,8-cineole. IC_{50} values for1,8-cineole was 1200 μg/ml. Thyme oil proved to reduce the viral load by >96%, whereas all monoterpenes by >80% ($p < 0.05$)	[69]
	in vitro	HIV-1 in HeLa HL3T1 cell line	Not defined	Essential oil	7.5–240 μg/ml	Neomycin, cisplatin	Possible alteration in the structure of Tat/TAR-RNA complex	EMSA showed a notable inhibitory potential of oil (3–6 μg/ml), compared to the control in case of Tat/TAR-RNA complex inhibition. Reduction activity test against Tat-induced HIV-1 LTR transcription resulted in RT_{50} = 0,83 μg/ml, a notable inhibitory potential which reduced viral transcription to 52% ($p < 0.05$)	[70]

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Table 3 (*continued*)

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
	in vitro	HIV-1 subtype A in PBMC cell line	Dried plant	Methanol extract	10, 100, 200, 800 and 1600 μg/ml	DMSO, Zidovudine	Not defined	The cytotoxicity value (CC_{50}) on PBMC was found to be 200 μg/ml. Antiviral assay revealed EC_{50} value of >500 μg/ml. Mean fluorescent intensity (MFI) of the CD4+ expressions were found to be 22.72 in PBMC ($p < 0.05$)	[71]
Antidiabetic activity	*in vitro*	Inhibition of α-glucosidase and α-amylase enzymes	Not defined	Aqueous, methanol and ethanol extracts	4, 8, 15, and 20 μg/ml	Acarbose	Not defined	Methanol extract resulted in maximum inhibition of α-glucosidase (IC_{50} 4.35, 22.04, 30.77, 43.13), though less compared to Acarbose (IC_{50} 16.11, 44.6, 53.03, 63.70). Similarly, α-amylase got reduced maximally by the same extract (IC_{50} 6.39, 11.47, 17.01, 22.93), less compared to Acarbose (IC_{50} 12.37, 25.16, 36.08, 44.97)	[72]
Anxiolytic activity	*in vivo*	Elevated plus-maze (EPM) rat model	Dried plant	Aqueous extract	50 mg/kg, 100 mg/kg, and 200 mg/kg	Saline fed groups	Possible relation with antioxidant activity of phytochemicals	The aqueous extract exhibited a significant increase in rat movement into the open arms at 100 mg/kg ($p < 0.05$) and 200 mg/kg ($p < 0.01$).	[74]
UV-protective activity	*in vitro*	Human skin cells	Not defined	Aqueous extract, thymol	1.82 μg/ml extract and 1 μg/ml thymol	Normal cells without UV treatment, but with extract treatment	Reduction of ROS induced DNA damage, Possible involvement of polyphenols in protectivity	Aqueous extract of thyme leaf (1.82 μg/ml) and thymol (1 μg/ml) reduced the release lactic acid dehydrogenase (LDH), in cultured skin cells treated with UV rays. Cell proliferation was observed in thyme pre-treated skin cells in accordance with control, along with the reduction in DNA damage ($p < 0.01$)	[75]
Anthelminthic activity	*in vitro*	Eimeria spp. oocysts from Turkey fowls	Not defined	Essential oil	0, 1, 2, 4, 8, 10, 20, 40, 80, and 800 mg/ml	Ammonia and diclazuril	Not defined	Thyme oil showed significant anti-helminthic activity against 4 species of *Eimeria* spp. at IC_{50} 53.42 mg/ml for 5×10^4 oocysts ($p < 0.05$)	[40]

(*continued on next page*)

Table 3 (*continued*)

Pharmacological Activity	Type of Study	Models used	Plant part/material	Type of extract/ compound	Doses used	Controls	Possible/reported mechanisms	Results	References
Anti-anti-alzheimer's activity	*in vivo*	Acetylcholine esterase and nicotinic acetylcholine receptor in *C. elegans* nematode model	Aerial parts and leaves	Essential oil	10, 20, 40, 60, 80, and 100 ppm	10% DMSO	Upregulation of unc-17, unc-50, and cho-1 genes by ρ-Cymene	Enhancement of the nicotinic acetylcholine receptor activity, upregulation of unc-17, unc-50, and cho-1 genes at 40 and 60 ppm ρ-Cymene was attributed for gene upregulation activity along with downregulating ace-1 and ace-2 at 20 and 100 ppm ($p < 0.05$). Thymol and γ-terpinene enhanced synaptic acetylcholine levels in combination (40 ppm)	[76]
Anti-osteoporotic activity	*in vivo*	Rat model with low calcium intake	Dried leaves	Leaf powder	5% w/w	Standard diet + normal calcium (Ca 0.5% w/w), standard diet + low calcium (Ca 0.1% w/w), Thyme powder (5% w/w) + low calcium (Ca 0.1% w/w)	Possible promotion of calcium resorption in the gut	Significant increase in the bone mass (2.93 g/kg), length 32.8 mm), and density (0.13 g/cm^2), compared to low calcium diet control (2.46 g/kg, 32.2 mm, and 0.09 g/cm^2, respectively) ($p < 0.05$)	[77]
Anti-pulpotomy activity	*in vivo*	Formocresolpulpotomy in humans	Not defined	Ethanolic extract	Suitable consistency	Formocresol	Not defined	Thyme ethanolic extract along with zinc oxide reduced pain and tenderness, Enhanced bone and root resorption. Clinical and radiographic evaluations showed 94.4% and 88.2% success, respectively with no statistical significance compared to the control, formocresol 88.2% ($p > 0.05$)	[78]

Table 4. Summary of clinical trials of *T. vulgaris* preparations.

Pharmacological activity	Type of study	Models	Plant part/material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
Adipogenetic and anti-aging	*in vitro*	Humans	Leaves and flowers	ThymLec gel 2% prepared from leaf and flower extract (1.0%–3.0%) + water, propanediol, glycerine +5.0%–13.4%) lecithin (additive) + benzyl alcohol, potassium sorbate, tocopherol (preservatives)	2 mg/cm^2 of the facial skin applied with 2% concentration (20 mg/g w/w), gently spreading, twice a day (morning and evening).	Placebo (formulation containing the same vehicle of ThymLec gel) and Benchmark 2%	ThymLec topical application leads into the adipogenesis and lipid production, further augmenting cell volume and better remodelling of face oval features. ThymLec modulates the PPAR-γ signalling pathway, increasing adiponectin production and adipocyte lipid accumulation.	Reduction of area (7.0%), depth, and length (10.2%) of the perioral wrinkles on day 60, whereas benchmark produced a reduction of 5.4% and 7.5%, respectively in case of humans. Length of the nasolabial lines (8.9%), Crow's feet wrinkles' area (8.9%), breadth (3.9s%), and length (11.1%) were also decreased. Face oval remodelling evaluation resulted in the reduction of total face volume (4.9 fold) in comparison with benchmark on day 60. *In vitro* adiponectin synthesis increased in 3T3-L1 embryonic fibroblasts treated with ThymLec 2% (122% at 0.0195% concentration) and (137% at 0.039% concentration).	[79]
Anti-dysmenorrhea	*in vitro*	Humans	Not defined	Essential oil	25 drops of essential oil	Ibuprofen, Placebo (Ibuprofen 200mg + oil 25 drops)	Anti-prostaglandin and antispasmodic activity of *T. vulgaris* L.	Pain intensity mean values reached lowest point (6.57–1.14 VAS) in case of oil consumers' group in comparison with placebo (6.13–3.45) and ibuprofen (5.3–1.48) ($p < 0.05$). No significant results were obtained in case of bleeding control by both oil and placebo. Oil was able to reduce clinical symptoms like lower abdominal pain, nausea, mood swing, and fainting after 48 h of consumption ($p < 0.05$).	[80]

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Table 4 (*continued*)

Pharmacological activity	Type of study	Models	Plant part/material	Type of Extract/ compound	Doses	Controls	Mechanisms	Results	References
Anti-bronchitis	*in vitro*	Humans	Whole plant extract	Dry powder extract	160 mg oral dose along with 60 mg primrose (*P. vulgaris*) root extract for 11 days	Placebo tablet without ingredients	Not defined	The extract reduced the cough symptoms on day 9 with an efficacy of 73.7% in comparison with placebo (day 11; 57.8%) ($p < 0.0001$). Reduction of bronchitis severity score was observed only after 4 days in case of extract (-6.2) in comparison with placebo (-4.1) ($p < 0.05$). The extract also reduced minor symptoms like disturbance in sleeping, rising body temperature, chest pain, and difficulty in breathing.	[12]

6.5. Anti-cancerous activity

Cytotoxic activity of thyme oil was analysed against MCF7 (breast adenocarcinoma), HCT15 (colon carcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and NCI-H460 (non-small cell lung cancer) cell lines. *T. vulgaris* L. oil showedgrowth inhibition for all the cell lines tested at 76.02–180.40 μg/ml concentration (GI_{50}). However, thyme oil did not show any effect on non-tumour liver PLP cells, even at a high concentration of 400 μg/ml ($p < 0.05$) [65]. In case of THP-1 leukemia cell line, at a concentration of 100 μg/ml and >200 μg/ml, thyme oil prevented the proliferation [66]. Apart from the oil, thymus extracts also proved to be effective against lung cancer cells. In a study, H460 lung cancer cell line was found to be sensitive at 0.11% of hydroalcoholic extract ($p < 0.05$) and downregulated NF-κB p65 and NF-κB p52 proteins along with the reduction of IL-1β and IL-8 gene expression in LPS model. However, there was no cytotoxicity reported within a concentration range of 0.04–0.60% [21].

In an *in vivo* study, administration of dried *T. vulgaris* L. powder to mammary carcinoma rat and 4T1 mouse models led to a remarkable reduction in the volume of 4T1 tumours by 85% at 1% concentration. In the rat model, the same concentration decreased the tumour frequency by 53% compared with that of the control, besides suppressing the genes associated with tumour inducing properties. This property is attributed to the resultant upregulation of caspase-2 and caspase-3 enzymes, along with bcl-2 and Bax proteins that result in cell apoptosis. Results were in accordance with the controls used, and these results were significant ($p < 0.05$ [67].

It was noteworthy to find that thyme oil and extracts show a notable cytotoxic effect on tumour cells, but not on normal human cells. A study conducted by Deb et al. (2011) showed the effect of thymol on HL-60 acute promyelotic leukemia cells. Thymol showed no cytotoxic effect on human peripheral blood mononuclear cell (PBMC) at 5 and 25μM concentrations. However, extensive cytotoxicity was observed at >50 μM, after 24 h [68]. Heidari et al. (2018) employed a different approach, where both the plant extract and synthesized silver nanoparticles from the plant extract were evaluated against T47D human breast cancer cells. T47D cells showed a high sensitivity towards nanoparticles (90%) when compared with that of the extract (75%). T47D cells treated with nanoparticles showed 18.40% early and 0.69% late apoptosis with a varying IC_{50} concentrations (12.5–100 μg/ml) ($p < 0.05$), whereas in case of plant extract, 15.67% early and 1.70% late apoptosis was observed [47].

The selectiveness of thyme oil and other extracts towards tumour cells needs to be studied, for the possible presence of molecular pathways that mediate the anti-cancerous effect. The mechanisms of *T. vulgaris* L. fractions on different cell lines need to be elucidated with respect to biochemical and physiological changes occurring within the proliferating cell. These findings may serve as key sources during drug development process. Most of the studies have attributed the anti-proliferative activity to the monoterpenes, carvacrol and thymol that are predominantly present in thyme oil [66, 68]. According to Aazza et al. (2014) carvacrol and thymol possess higher anti-cancerous potential than p-cymene and borneol [66]. Thus, isolation and identification of individual components for anti-cancerous potential needs to be done. Although Deb et al. (2011) have deduced the mechanism of thymol in causing damage to the cancer cell via inducing the activity of apoptosis inducing factor (AIF) [68], much remains unknown about the other compounds. In a new approach, nanoparticles have induced better cell apoptosis compared with that of the plant extract, but with more cytotoxic effect [47]. Therefore, studies need to decipher the appropriate doses for consumption, prior to the complete decoding of the mechanism of action. Accurate determination of these doses using human cell lines and animal models could be useful to conduct clinical trials for drug development.

Also, effect of the plant fractions needs to be correlated with the expression of oncogenes, which may deduce druggable targets ensuring a check on its toxicity. For example, thymol showed higher cytotoxic effects in animal models at >50 μM, compared with the other extracts [68].

It becomes essential to focus on the other compounds present in the essential oil with lower toxicity level. Minimum toxicity levels have been reported with plant extracts and dried powder, which can be utilized for further analysis. Furthermore, much remains unknown about cancers like Hodgkin and non-Hodgkin's lymphomas, Kaposi sarcoma and leukemia. These cancer models along with the other rare cancer types also need to be evaluated.

6.6. Antiviral activity

Thyme oil in the form of vapour and liquid was found to be effective against the influenza virus. However, partial activity was observed in vapour phase, whereas the liquid phase at 3.1 μl/ml concentration completely inhibited the viral growth, which was better than that of control used (canola oil). In addition, the researchers also evaluated the effects on the principle external proteins of the virus, namely the hemagglutinin (HA) and neuraminidase (NA), where significant inhibition of HA was observed. In addition, the TC50 value, which is 50% reduction of the culture, was found to be 14.34 μl/ml ($p < 0.05$) [33].

Apart from influenza, thyme oil was found to be effective on causative viruses of sexually transmitted diseases (STDs) like herpes simplex virus (HSV) and human immunodeficiency virus 1 (HIV-1). HSV possesses two antigenic types, type 1 (HSV-1) and type 2 (HSV-2), resulting in flu like symptoms in humans. Thyme oil along with the major monoterpene compounds α-terpinene, terpinen-4-ol, α-terpineol, α-pinene, p-cymene, thymol, citral and 1,8-cineole were analysed for their antiviral activity. RC-37 kidney cells were used for non-cytotoxic dose determination, which ranged between 20 μg/ml for citral and 1250 μg/ml for 1,8-cineole. IC_{50} values for 1,8-cineole was 1200 μg/ml. Thyme oil proved to reduce the viral load by >96%, whereas all monoterpenes by >80% ($p < 0.05$) [69].

The human immuno deficiency virus is another STD which has no available vaccine yet. A study conducted by Feriotto et al. (2018) targeted the Tat protein that aids in the transcription of the viral genome. This study evaluated the interaction of the essential oil from *T. vulgaris* L. with the transcription of the Tat/TAR-RNA complex as well as the Tat-induced HIV-1 long terminal repeat (LTR) [70]. An electrophoretic mobility shift assay (EMSA) for this complex showed a notable inhibitory potential (3–6 μg/ml), compared with that of the control. Similarly, a reduction activity test against the Tat-induced HIV-1 LTR transcription resulted in RT_{50} = 0,83 μg/ml, a notable inhibitory potential which reduced viral transcription to 52% ($p < 0.05$) [70]. Similarly, the methanol extract of the plant was evaluated on the infected PBMC cells with HIV-1 subtype A. The cytotoxicity value (CC_{50}) on PBMC was found to be 200 μg/ml. Further, the antiviral assay revealed EC_{50} value of >500 μg/ml. The study also focused on CD4+ expressions, where mean fluorescent intensity (MFI) of the cells was found to be 22.72 in PBMC ($p < 0.05$) [71].

The studies have primarily focused on determining cytotoxicity values using animal models, which may not give an accurate account in case of human trials [69, 71]. Thus, it becomes essential to conduct experiments using human cell lines. Human cells can be infected *in vitro*, subsequently treated with different extracts, oil, and individual compounds to provide conclusive evidence of its effects on human system. Effect of plant fractions on viral genome and protein synthesis needs to be studied along with the gene expression levels. Using bioinformatic tools like molecular docking to study the binding efficiency of plant-based compounds to viral proteins could be a feasible approach. For example, Feriotto et al. (2018) have deduced the role of TAT/TAR-RNA complex in the virulence of HIV-1. The same complex could be assessed in terms of its interaction with the plant phytochemicals [70]. Including HIV-1, diseases like influenza and Herpes are often difficult to identify because they are asymptomatic and have an extended incubation period. Herpes has a substantial effect on skin, which could be evaluated by the application of thyme oil. As the viruses affect targeted organs, an organ-targeted delivery of the modified plant fraction could be studied in a dose-dependent manner.

6.7. Other pharmacological activities

Apart from major pharmacological properties, *T. vulgaris* L. is reported to comprise a few other properties which are yet be focused on. Studies are yet to be conducted to assess the antidiabetic and anti-hyperglycemic activities regarded as the indispensable properties of a medicinal plant. A study by Aljarah and Hameed (2018) depicts the *in vitro* inhibition of α-glucosidase and α-amylase enzymes known to be the potential targets of antidiabetic drugs. Out of aqueous, methanol and ethanol extracts at different concentrations (4, 8, 15, and 20 μg/ml), methanol extract resulted in maximum inhibition of α-glucosidase (IC_{50} 4.35, 22.04, 30.77, 43.13) and α-amylase (IC_{50} 6.39, 11.47, 17.01, 22.93), yet lesser than the standard drug acarbose [72]. As stress and anxiety levels reported to play a crucial role in diabetes [73], *T. vulgaris* L. extract was also evaluated for its impact on anxiety levels in *T. vulgaris* L. on elevated plus-maze (EPM) rat model. The aqueous extract exhibited a significant increase in rat movement into the open arms at 100 mg/kg ($p < 0.05$) and 200 mg/kg ($p < 0.01$) [74].

Further, thyme oil and extracts were evaluated for their protectant ability against UV-A and UV-B rays, by inhibiting the proliferation of skin cells leading to cancer. Aqueous extract of *T. vulgaris* L. leaf (1.82 μg/ml) and thymol (1 μg/ml) reduced the release of lactic acid dehydrogenase (LDH), in cultured skin cells treated with UV rays. Significant cell proliferation was observed in *T. vulgaris* L. pre-treated skin cells in accordance with that of the control used, along with the reduction in DNA damage ($p < 0.01$) [75]. In addition, an *in vitro* evaluation of thyme oil showed a significant anti-helminthic activity against 4 species of *Eimeria* spp. at IC_{50} 53.42 mg/ml for 5×10^4 oocysts ($p < 0.05$). These parasites affect poultry and cattle, reducing the yield [40].

Cholinergic affliction is found in Alzheimer's disease, a progressive neurodegenerative disorder involving the death of cholinergic neurons. Administration of thyme oil to *C. elegans* led to an enhancement of the neurotransmission by regulating synaptic acetylcholine levels [76]. Enhancement of the nicotinic acetylcholine receptor activity is also observed because of the upregulation of unc-17, unc-50, and cho-1 and genes at 40 and 60 parts per million (ppm). p-cymene, a monoterpene present in thyme extract, was attributed for the gene upregulation activity along with a corresponding downregulation of ace-1 and ace-2 at 20 and 100 ppm ($p < 0.05$). Interestingly, thymol and γ-terpinene enhanced synaptic acetylcholine levels in combination (40 ppm) but failed to do the so when administered individually [76].

Aging, a condition characterized by a reduced bone density, can be treated by increasing calcium uptake in the body. Elbahnasawy et al. (2019) conducted a study depicting the effect of *T. vulgaris* L. powder on rats with low calcium intake. The study resulted in a significant increase in the bone mass (2.93 g/kg), length 32.8 mm), and density (0.13 g/cm^2), compared with that of the low calcium diet control (2.46 g/kg, 32.2 mm, and 0.09 g/cm^2, respectively) ($p < 0.05$). This study proved the potential of *T. vulgaris* L.in the treatment of osteoporosis and other bone related diseases [77]. *T. vulgaris* L. is also effective on dental caries and proved its efficacy over the formed ocresolpulpotomy, due to profound antibacterial properties. *T. vulgaris* L. ethanolic extract along with zinc oxide was used in patients with dental caries. Clinical and radiographic evaluations showed 94.4% and 88.2% improvement, respectively with no statistical significance compared to that of the standard drug formocresol 88.2% ($p > 0.05$) [78].

With the studies depicting different pharmacological potentials of *T. vulgaris* L., it becomes evident that more studies need to be conducted using different approaches. For example, antidiabetic study conducted by Aljarah and Hammed (2019) using *in vitro* enzyme inhibition studies lack the usage of animal models and human cell lines [72]. In addition, a dose-dependent evaluation of the plant extracts is essential in order to understand the optimum dosage and toxicity. Analysis of binding of

individual compounds could be done using molecular docking, which can become a pavement for drug development process. Also, studies need to focus on feasible experimental designs, which can give better yields, less toxicity and more pharmacological effect. If more studies with novel pharmacological models are available, one can compare these studies and suitable methods can be assayed to develop a specific dosage and formulation. The anxiolytic EPM model by Komaki et al. (2016) has highlighted the need for further evaluation of the mechanism behind the observed effect [73]. More elucidations need to be done with respect to the brain activity, secretion of hormones, and blood pressure upon the administration of different doses of *T. vulgaris* L. extracts. Further, a study conducted by Elbahnasawy et al. (2019) has discussed the anthelminthic activity of *T. vulgaris* L. *in vitro* that could be better represented using turkey as an animal model, instead of isolating the parasite from its feces [74]. Furthermore, the evaluation of *T. vulgaris* L. activity on dental caries need to be conducted using anti-infective or antibacterial assay primarily because the caries is principally caused by bacteria. In total, these studies depict *T. vulgaris* L.as a storehouse of different pharmacological properties attributed to the presence of various phytochemical compounds. Thus, isolation, identification and determination of the pharmacological properties of individual compounds needs to be focused. A summary of the pharmacological properties of different extracts and isolated compounds of *T. vulgaris* L. is given (Table 3). With this, one can understand the pharmacological evaluations done so far, and can further refine the research methodologies.

7. Clinical trials of *T. vulgaris* L. preparations

Despite many explorations in the aspects of pharmacological efficiency, very little data are available on the clinical trials of *T. vulgaris* L.-based products. Our literature survey could find only few studies evaluating the pharmacological potential of *T. vulgaris* L.-based products using humans as study models [79, 80]. These clinical trials used different forms of *T. vulgaris* extracts and have resulted in the amelioration of the patients'/volunteers' health conditions (Table 4). Chemical characterization of these herbal products is yet to be done, which could decipher the specific action of phytochemicals involved in the pharmacological activity. Recently, a study by Caverzan et al. (2020) prepared a gel named ThymLec gel 2%, which was used as a phytocosmetic to improve facial health conditions [79]. A preparation from leaf and flower extract (1.0%–3.0%) + water, propanediol, glycerine +5.0%–13.4%) lecithin (additive) + benzyl alcohol, potassium sorbate, tocopherol (preservatives), application of this gel2 mg/cm^2 on female facial skin with 2% concentration (20 mg/g w/w) twice a day (morning and evening) resulted in the reduction of area (7.0%), depth, and length (10.2%) of the perioral wrinkles on day 60, whereas the benchmark produced a reduction of 5.4% and 7.5% of the same parameters, respectively. The length of the nasolabial lines was also decreased (8.9%). Similarly, area (8.9%), breadth (3.9%), and length (11.1%) of the crow's feet wrinkles were decreased with a corresponding reduction in the smile lines by 6.9%, 4.1%, and 10.1%, respectively. Face oval remodelling evaluation using ThymLec 2% resulted in the reduction of total face volume (4.9 fold) in comparison with benchmark on day 60. *In vitro* adiponectin synthesis was significantly increased in cultured 3T3-L1 embryonic fibroblasts treated with ThymLec 2% (122% at 0.0195% concentration) and (137% at 0.039% concentration). This was 87% higher than the benchmark used. A dose-dependent treatment of ThymLec 2% resulted in the expression of PPAR-γ mRNA levels, in comparison with that of the control groups. ThymLec topical application led to adipogenesis and lipid production, further augmenting cell volume and better remodelling of face oval features. ThymLec modulates the PPAR-γ signalling pathway, increasing adiponectin production and adipocyte lipid accumulation [79.

In another trial, which was focused on the amelioration effects of *T. vulgaris* L. on dysmenorrhea (painful menstruation in females), essential oil (25 drops) was investigated for its pain relieving properties [80]. Pain intensity during menstruation was measured in visual analogue scale (VAS) on a 10 cm line before and after administration of *T. vulgaris* L. essential oil. Pain intensity mean values reached the lowest point (6.57–1.14) in case of oil consumers' group in comparison with that of the placebo (6.13–3.45) and ibuprofen (5.3–1.48) ($p < 0.05$). However, no significant results were obtained in case of bleeding control by both oil and placebo (Ibuprofen 200mg + oil 25 drops). In addition, the oil was able to reduce clinical symptoms like lower abdominal pain, nausea, mood swing, and fatigue after 48 h of consumption ($p < 0.05$). Around 71.4% of the volunteers marked *T. vulgaris* L. oil as excellent whereas ibuprofen (28.0%) and placebo (14.3) were found to be underscored. Here, anti-prostaglandin and antispasmodic activity of *T. vulgaris* L. was attributed [80].

The use of *T. vulgaris* L. in the treatment of respiratory ailments [37], was supported by a clinical trial investigating a combination of *T. vulgaris* and *Primula vulgaris* (primrose, common thyme) dry powder extract on bronchitis patients. The combination (oral dose of 160 mg thyme and 60 mg primrose root extract for 11 days) reduced the cough symptoms on day 9 with an efficacy of 73.7% in comparison with that of the placebo (day 11; 57.8%) ($p < 0.0001$). Reduction of bronchitis severity score was observed only after 4 days in case of thyme and primrose treatment group (-6.2) in comparison with the placebo (-4.1) ($p < 0.05$). Extract was well tolerated in most of the patients (183) with only 1.7% adverse effects reported with 97.8 % patients rating this combination as preferable. The extract also reduced minor symptoms like disturbance in sleep, rising body temperature, chest pain, and difficulty in breathing [12]. However, no underlying mechanisms were decoded during this study. Therefore, understanding the mode of action along with product formulation with lesser adverse effect and affordability are to be focused. Research also needs to be focused on revealing the chemical components of such herbal products made.

8. Toxicology

T. vulgaris L. is considered as a culinary herb and has been extensively used since time immemorial in Europe and Mediterranean. In all the records of ethnomedicinal properties, there is no mention of toxic effects. Moreover, it has been described as the most used herb by the physician Dioscorides in the 2nd century [8]. In support of this, present-day studies report no cytotoxic activities of either the extracts or oil. A 28-day oral dose toxicity assessment of thyme oil in rats was conducted in single (2, 000 mg/kg) and repeated dose (100, 250, and 500 mg/kg/day) methods, where no signs of toxicity were detected. Even with the administration of 500 mg/kg/day, the body weight was found to be altered, yet with no toxicity observed even on 28th day [81]. In support to this, a study conducted by Benourad et al. (2014) assessed the activity of thyme essential oil and found no histopathological changes in the rat liver and kidney, except a few metabolic changes including increase in urea nitrogen, creatinine and uric acid levels. Besides this, alterations in glycolysis, β-oxidative pathways, Kreb's cycle were also observed. In total, this study revealed that injection of repeated doses of thyme oil results in a few intermediary metabolic disturbances with no cytotoxic activities [82]. Several studies conducted on cancer cell lines reported the cytotoxic effect on tumour cells. However, thyme oil did not show any effect on non-tumour liver PLP cells, even at the high concentration of 400 μg/ml [66]. Even thymol is reported with no cytotoxic effect on normal human cells except >50 μM [68]. From these studies, it becomes evident that the *T. vulgaris* L. plant extracts and oil possess no cytotoxic activity though there are reported cases of mild metabolic changes.

9. Perspectives and projections

Based on the available literature and gaps present with respect to ethnopharmacology, pharmacology, and phytochemistry of *T. vulgaris* L., a few perspectives and future projections could be drawn out. Though a tremendous amount of ethnobotanical literature is present, it lacks

specifications in terms of plant material, plant parts, and extracts used in ancient times. With these foundational evidences, concrete modern-day pharmacological approaches could be used, thereby supporting the vital claims by the previous generation that in turn could be used in drug discovery. Pharmacological findings need to be supported by the mechanisms. The action of plant extracts and individual compounds need to be defined at the molecular level to find more druggable targets, which can further be used to design a specific drug. Moreover, these studies have used multicomponent assays with varying concentrations, which complicate the determination of specific dose. Thus, qualitative and quantitative assays using individual compounds in a dose-dependent manner and a combination approach could reduce the burden of multifactorial results. Isolated compounds from the plant extracts could be further modified according the requirement based on cytotoxic evaluations done in primary screening. Such compounds could be analysed *in silico* for effective binding and inhibitory effect on specific component or any part of the identified target. Further, the bioavailability of the compounds could be dealt to modify the compound to match the physiological conditions of the target organ. Usage of *in silico* methods like molecular modelling and docking can save a lot of animals, which are used in laboratories.

Many of the studies quoted in this review need to be modified in terms of their methodology. Antimicrobial evaluations need a reformation, where the zone of inhibition could be excluded. Metabolic approaches like enzyme inhibition, protein inactivation, and interference in DNA and protein biosynthesis could be carried out. These mechanisms support the specific drug discovery process. In case of antioxidant activity, conventional assays like DPPH, ABTS, and FRAP need to be excluded, as advancements including evaluation of cytokine factors and antioxidant enzymes have been a major concern in recent years. Herein, the use of *in vivo* approach in support to the *in vitro* findings needs to be focused. Evaluations require animal model and cell lines with drug-induced autoimmunity. As the inflammation is concerned with several signalling pathways and immune cells, the fate of these cells and pathways need to be studied upon the introduction of plant fractions. Anticancerous studies need to include animal model and more *in vivo* approaches than cell lines, along with a dose-dependent approach using individual compounds.

These suggestions are utmost necessary to enhance the pharmacological studies associated with the plant products. Following up these protocols will help in the generation of data from phytochemicals, which can be transferred into clinical trials to get a completely processed plant product. Furthermore, high quality, advanced phase, randomized, placebo-controlled, multi-centred, randomized, and double-blind clinical trials could be done.

10. Conclusion

The present-day proximate analysis of *T. vulgaris* L. supports the use of the plant as an important ingredient in food since the Egyptian era. Alongside biomolecules, the rich diversity of phytochemicals present in the *T. vulgaris* L. plant may be accountable for major health benefits. The ethnomedicinal uses of the plant have revealed the importance in treating various diseases. Several studies conducted *in vitro* and *in vivo* using cell lines and animal models with induced pathological conditions have proved the efficiency of the plant as a therapeutic agent. *T. vulgaris* L. is found to be effective against both infectious and foodborne pathogens. Both the oil and extract of the plant were evaluated for their effects on inflammation and cancer. Along with the proven pharmacological aspects, there are several activities that are yet to be reported. For example, the literature survey could find only 2 studies with significant results on anti-diabetic potential of the plant. Furthermore, inhibition of acetylcholine esterase could be assessed for more physiological aspects, especially with diabetes. The extensive inhibitory effect on foodborne and oral pathogens has proved its possible use as a preservative agent and an important drug for dental caries, respectively. Meanwhile, *T. vulgaris* L. also possesses anti-helminthic property, which can make it a prominent therapeutic agent against parasitic infections. Also, a great amount of work needs to be done with respect to its skin protection activity. The promising evidence on the cell proliferation post-exposure to UV rays suggests its clinical trials for establishing a potent product. Pre-clinical studies at a large scale including *in vivo* cytotoxicity assays are suggested for the evaluation of accurate dosage levels of different plant fractions. Pharmacokinetics and pharmacodynamics of the compounds need to be focused. In addition, interactions with dietary molecules and drugs need to be assessed for the development of nutraceuticals. The pharmacological analysis at the genetic and proteomic level, along with commercially available drugs would determine the beneficial and harmful effects of the plant extracts or individual compounds. In total, *T. vulgaris* L. can open the doors to the development of many prominent therapeutic agents with diverse nature of applications to resolve several health maladies.

Declarations

Author contribution statement

Ramith Ramu; Shashank M Patil; Prithvi S Shirahatti: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Raghavendra G. Amachawadi; Chandan Shivamallu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

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