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Review

An Overview of the Biological and Pharmacological Effects of Thymol

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Abstract

Background: Thymol is a naturally occurring monoterpenoid phenol mainly derived from *Thymus* species and is known for its wide range of biological activities. This study aims to provide a comprehensive and critical overview of the biological and pharmacological effects of thymol, with particular emphasis on its mechanisms of action and therapeutic potential. Methods: Relevant data from *in vitro* studies, animal models, and recent advances in drug delivery systems were analyzed, focusing on pharmacological activities, molecular mechanisms, and safety profiles. Results: Thymol exhibits antimicrobial activity primarily by disrupting microbial membranes and interfering with intracellular processes. Its antioxidant and anti-inflammatory effects involve modulation of key signaling pathways, including NF-κB and PI3K/Akt. It also shows antinociceptive and smooth modulatory properties, suggesting potential applications in pain management and gastrointestinal disorders. Advanced delivery systems, such as nanoemulsions and liposomes, improve its stability and bioavailability. Toxicological studies indicate a generally favorable safety profile, although dose-dependent effects require careful consideration. Conclusion: Thymol is a multifunctional compound with promising pharmacological applications. However, further clinical studies are necessary to confirm its efficacy and safety in humans.

Keywords: Antimicrobial activity; Antioxidant properties; Anticancer activity; Gastroprotective effects; Anti-inflammatory action

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1. Introduction

Thymol (2-isopropyl-5-methylphenol) is a naturally occurring monoterpenoid phenol pre-dominantly associated with essential oils extracted from aromatic and medicinal plants belonging to the Lamiaceae family (PubChem., 2023). Its abundance is particularly notable in plants of the genus *Thymus*, especially *Thymus vulgaris*, where its concentration may vary considerably according to chemotypic variation, ecological factors, and geographical origin (Salehi *et al*., 2018). Such variability not only influences the aromatic profile of the essential oil but also modulates its biological potency (Burt., 2004). Beyond *Thymus*, thymol is also detected in related genera such as *Origanum*, contributing to their distinctive phytochemical and pharmacological characteristics (Skoula & Harborne., 2002).

From a structural standpoint, thymol is characterized by a phenolic hydroxyl group linked to a substituted aromatic ring bearing isopropyl and methyl moieties (PubChem., 2023). This configuration confers pronounced lipophilicity while preserving hydrogen-donating ability, a dual property that underlies many of its bioactivities. Its affinity for lipid environments facilitates integration into biological membranes, where it can alter permeability and disturb membrane-associated functions. Simultaneously, the phenolic structure enables participation in redox reactions, supporting its role in oxidative stress modulation.

Traditionally employed as an antiseptic and preservative, thymol has long been incorporated into pharmaceutical and food formulations (Burt., 2004). Its inclusion in oral hygiene products and topical preparations reflects well-established antimicrobial properties. However, contemporary pharmacological investigations have revealed a broader and more complex biological profile than previously appreciated. Experimental evidence indicates that thymol exerts antibacterial and antifungal effects through membrane disruption and interference with essential metabolic pathways (Marchese *et al*., 2016).

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In parallel, antioxidant activity attributed to thymol extends beyond simple radical scavenging (Salehi *et al*., 2018). Modulation of lipid peroxidation processes and regulation of intracellular oxidative balance suggest a more integrated mechanism involving redox-sensitive signaling networks. Its anti-inflammatory potential has been linked to the regulation of transcription factors such as NF-κB (Hosseini *et al*., 2021). Furthermore, emerging data propose that thymol can interfere with proliferative signaling and promote programmed cell death under specific experimental conditions.

Collectively, these converging lines of evidence position thymol as a multifunctional phytochemical with translational relevance. Nevertheless, despite substantial preclinical documentation, important questions remain regarding dose optimization, pharmacokinetics, and clinical validation. Therefore, the main objective of this review is to provide a comprehensive and critical synthesis of the biological and pharmacological effects of thymol, with emphasis on its mechanisms of action and therapeutic potential.

2. Chemical properties and sources

Thymol is a naturally occurring phenolic monoterpene characterized by the molecular formula $C_{10}H_{14}O$ and a molecular weight of 150.22 g/mol (PubChem., 2023; Marchese *et al*., 2016). Structurally, it originates from the biosynthetic assembly of two isoprene units and contains a hydroxyl group attached to an aromatic ring, which classifies it among phenolic monoterpenoids (Burt., 2004; Marchese *et al*., 2016). The presence of this aromatic phenolic structure strongly influences its physicochemical properties and biological behavior.

From a physicochemical perspective, thymol exhibits pronounce lipophilic characteristics. Its octanol–water partition coefficient (log P), generally reported between 3.0 and 3.5, indicates a strong tendency to associate with lipid environments rather than aqueous media (PubChem., 2023; Nostro & Papalia., 2012). Consistent with this property, thymol shows limited solubility in water while remaining readily soluble in many organic solvents (Burt., 2004). Such hydrophobicity facilitates its incorporation into phospholipid bilayers and promotes its accumulation within biological membranes (Ultee *et al*., 2002; Lambert *et al*., 2001).

In addition to its lipophilic nature, thymol displays moderate volatility, with a boiling point of approximately 232–233 °C and a measurable vapor pressure at ambient temperature (PubChem., 2023). This property explains its presence in essential oil vapors and contributes to its antimicrobial activity not only in liquid systems but also in the vapor phase (Burt., 2004).

Naturally, thymol occurs as a major component of several essential oils, particularly those produced by plants of the Lamiaceae family. In *Thymus vulgaris* (thyme), thymol may represent between 20% and 60% of the total essential oil composition, although higher concentrations have been reported in specific chemotypes (Burt., 2004; Marchese *et al*., 2016). The exact proportion can vary considerably depending on genetic factors, environmental conditions, and extraction techniques (Marchese *et al*., 2016). Thymol is also present in the essential oil of *Origanum vulgare* (oregano). However, in this species, carvacrol is frequently the dominant phenolic compound, while thymol levels vary according to chemotype and geographic origin (Lambert *et al*., 2001; Nostro & Papalia., 2012).

The biological activity of thymol is largely associated with its interaction with cellular membranes. Its amphipathic molecular structure enables efficient interaction with lipid bilayers: the hydrophobic aromatic ring and alkyl substituents facilitate insertion into the lipid core of the membrane, whereas the phenolic hydroxyl group can interact with polar phospholipid head groups through hydrogen bonding (Ultee *et al*., 2002; Lambert *et al*., 2001). Experimental studies have shown that this interaction leads to increased membrane permeability, disruption of proton gradients, and leakage of intracellular components in bacterial cells (Ultee *et al*., 2002; Nostro & Papalia., 2012). Consequently, membrane destabilization is widely considered one of the principal mechanisms underlying the antimicrobial activity of thymol (Burt, 2004; Marchese *et al*., 2016).

3. Biological effects of thymol

Thymol is a phenolic monoterpene naturally present in significant amounts in the essential oils of several *Thymus* species. Because of its long-standing use in traditional medicine, particularly as an antiseptic and preservative, this compound has attracted increasing scientific attention in recent years. Current research highlights thymol as a molecule with a wide range of biological and pharmacological properties (Marchese *et al*., 2016; Salehi *et al*., 2018).

The biological activities associated with thymol are largely related to its chemical structure. The presence of a phenolic hydroxyl group combined with a hydrophobic aromatic backbone contributes to its lipophilic character and facilitates interactions with cellular membranes. These structural features also enable thymol to influence intracellular signaling pathways that regulate various cellular processes (Nagoor Meeran *et al*., 2017).

Experimental investigations have demonstrated that thymol possesses marked antimicrobial activity against numerous bacterial and fungal species (Ahmad *et al*., 2011; Marchese *et al*., 2016). In addition, it has been reported to exhibit strong antioxidant properties, mainly through its ability to scavenge reactive oxygen species and other free radicals (Salehi *et al*., 2018; Nagoor Meeran *et al*., 2017). Beyond these effects, several studies have indicated that thymol may exert anti-inflammatory and anticancer activities. These actions are thought to involve the modulation of molecular pathways associated with oxidative stress, inflammatory mediators, and apoptosis (Nagoor Meeran *et al*., 2017; Salehi *et al*., 2018; Sharifi-Rad *et al*., 2018). Overall, the available evidence suggests that thymol represents a multifunctional natural compound with considerable potential for applications in the pharmaceutical, food preservation, and biomedical fields.

3.1 Antimicrobial effects

Thymol is widely recognized for its broad antimicrobial properties, showing inhibitory effects against numerous bacteria, fungi, and certain viruses. These activities are largely associated with its phenolic structure and its

capacity to interact with and destabilize microbial membranes (Burt., 2004; Marchese *et al*., 2016). Experimental studies have reported that thymol is effective against several Gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus cereus*, as well as Gram-negative species such as *Escherichia coli* and *Salmonella enterica* (Lambert *et al*., 2001; Burt., 2004). In many cases, Gram-positive microorganisms appear more susceptible, which is commonly attributed to the absence of an outer membrane that in Gram-negative bacteria may limit the penetration of hydrophobic molecules (Burt, 2004). Reported minimum inhibitory concentration values generally fall within the range of 100–1000 µg/mL, although this range varies depending on the microorganism tested and the experimental design (Lambert et al., 2001).

At the cellular level, thymol mainly exerts its antimicrobial effect through disruption of membrane integrity. Because of its lipophilic aromatic structure, the compound can readily insert into the lipid bilayer, modifying membrane fluidity and permeability (Ultee *et al*., 2002). Therefore, intracellular components such as ATP and potassium ions may leak from the cell, leading to dissipation of the proton motive force and impairment of essential metabolic processes (Ultee *et al*., 2002; Lambert *et al*., 2001). The phenolic hydroxyl group also contributes to antimicrobial activity by facilitating hydrogen bonding and proton exchange across the membrane (Ultee *et al*., 2002).

In addition to its antibacterial effects, thymol has demonstrated antifungal activity against organisms such as *Candida albicans* and various filamentous fungi. In these organisms, the compound interferes with membrane organization, particularly by affecting sterol-dependent membrane stability, which ultimately leads to growth inhibition (Marchese *et al*., 2016). Several studies also indicate that thymol may act synergistically with other essential oil constituents, including carvacrol, as well as with certain conventional antibiotics. Such combinations can enhance membrane disruption and reduce the effective concentration required for antimicrobial activity (Nostro & Papalia., 2012). Overall, the antimicrobial action of thymol is mainly attributed to its ability to integrate into microbial membranes, causing structural damage, energy imbalance, and ultimately cell death (Burt, 2004; Marchese *et al*., 2016).

3.2 Antioxidant effects

thymol also displays considerable antioxidant activity, which is primarily linked to the presence of its phenolic hydroxyl group. This functional group can donate a hydrogen atom to neutralize reactive radicals, thereby interrupting oxidative chain reactions involved in lipid peroxidation (Nostro & Papalia., 2012; Marchese *et al*., 2016). In chemical assays such as DPPH and ABTS radical scavenging tests, thymol exhibits a clear dose-dependent antioxidant response, confirming its ability to act as an effective radical scavenger (Burt, 2004; Marchese et al., 2016). The stability of the phenoxyl radical formed after hydrogen donation is enhanced by resonance within the aromatic ring, which further contributes to its antioxidant capacity (Nostro & Papalia., 2012).

Evidence from biological models also supports these antioxidant properties. In experimental studies involving animal models, administration of thymol has been associated with reduced levels of malondialdehyde (MDA), a commonly used indicator of lipid peroxidation (Fachini-Queiroz *et al*., 2012; Marchese *et al*., 2016). In parallel, increased activity of endogenous antioxidant enzymes such as superoxide dismutase and catalase has been observed (Marchese *et al*., 2016). Because oxidative stress and inflammation are closely interconnected processes, the antioxidant properties of thymol are believed to contribute to its broader protective effects in metabolic and inflammatory conditions (Fachini-Queiroz *et al*., 2012). Through reduction of reactive oxygen species and stabilization of cellular membranes, thymol may therefore support cellular defense mechanisms against oxidative damage

3.3 Anti-inflammatory effects

A growing body of evidence indicates that thymol possesses anti-inflammatory activity in several experimental models. These effects appear to result from the modulation of key inflammatory mediators, including cytokines, prostaglandins, and nitric oxide (Marchese *et al*., 2016; Fachini-Queiroz *et al*., 2012). In animal models of acute inflammation, thymol treatment has been shown to reduce leukocyte migration and tissue edema, suggesting a significant attenuation of inflammatory responses (Fachini-Queiroz *et al*., 2012). This reduction is accompanied by decreased production of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β, both of which play central roles in the amplification of inflammatory cascades (Fachini-Queiroz *et al*., 2012; Marchese *et al*., 2016).

Thymol has also been reported to suppress nitric oxide production in activated macrophages by downregulating the expression of inducible nitric oxide synthase (iNOS) (Nostro & Papalia, 2012; Marchese *et al*., 2016). Since excessive nitric oxide generation contributes to oxidative and nitrosative stress, this inhibition provides an additional mechanism supporting the compound's anti-inflammatory effects. Moreover, thymol may interfere with prostaglandin synthesis through modulation of cyclooxygenase pathways (Fachini-Queiroz *et al*., 2012). Prostaglandins such as PGE2 are major mediators of pain, fever, and vasodilation during inflammation, and their inhibition can therefore contribute to the anti-edematous and analgesic effects observed in experimental studies. At the molecular level, several of these actions are associated with inhibition of the NF-κB signaling pathway, a transcription factor that regulates genes involved in inflammatory responses (Marchese *et al*., 2016). By suppressing this pathway, thymol reduces the expression of multiple inflammatory mediators simultaneously.

3.4 Other reported effects

Beyond the activities described above, thymol has also been investigated in several additional biological contexts, including anticancer, neuroprotective, insecticidal, and gastrointestinal applications.

3.4.1 Anticancer effects

Anticancer activity has been reported in various *in vitro* models, where thymol exhibits cytotoxic and pro-apoptotic effects in cancer cell lines such as breast, colon, and leukemia cells (Marchese *et al*., 2016). These effects have been associated with mitochondrial dysfunction, increased intracellular ROS production, activation of caspases, and induction of apoptosis. In addition, inhibition of cell proliferation and cell cycle arrest have been observed following thymol exposure (Nostro & Papalia., 2012). However, most available evidence remains limited to cell culture or animal studies, and clinical validation is still lacking.

3.4.2 Neuroprotective effects

have also been suggested in experimental studies. Through its antioxidant and anti-inflammatory properties, thymol may contribute to the reduction of oxidative damage in neural tissues (Fachini-Queiroz *et al*., 2012; Marchese *et al*., 2016). Some investigations further indicate that thymol can modulate neurotransmission, partly through interactions with GABA-A receptors, which may influence neuronal excitability and protective responses in models of neuroinflammation (Marchese *et al*., 2016)

3.4.3 Insecticidal effects

Thymol additionally shows significant insecticidal and acaricidal activity and has been investigated as a botanical pesticide (Burt., 2004). Its lipophilicity and volatility facilitate penetration through insect cuticles, while its interaction with cellular membranes and the nervous system contributes to its toxic effects against various pests and mites (Marchese *et al*., 2016).

3.4.4 Gastrointestinal effects

Gastrointestinal studies indicate that thymol may exert spasmolytic and gastroprotective actions. Experimental models suggest that it can reduce smooth muscle contraction in the intestine and may therefore alleviate gastrointestinal spasms (Fachini-Queiroz *et al*., 2012). Its antimicrobial activity may also influence gut microbial balance by limiting pathogenic bacteria. In addition, protective effects against experimentally induced gastric lesions have been reported, likely linked to its antioxidant and anti-inflammatory mechanisms (Marchese *et al*., 2016).

Overall, while these additional biological effects are promising, most available data are derived from *in vitro* or *in vivo* studies. Further clinical investigations are therefore necessary to determine the therapeutic relevance of thymol in humans (Marchese *et al*., 2016).

4. Mechanisms of action

The wide range of biological activities attributed to thymol arises from several interconnected biochemical and cellular mechanisms, which are responsible for their antimicrobial, antioxidant, anti-inflammatory, and analgesic effects.

4.1 Antimicrobial Mechanism

Thymol exhibits antimicrobial activity through a multifaceted mode of action that primarily involves disruption of microbial cell integrity and interference with essential physiological functions. Owing to its hydrophobic character, thymol readily associates with the lipid components of cell membranes, leading to structural disorganization and increased permeability, a widely reported phenomenon in studies on essential oil constituents (Burt., 2004; Ultee *et al*., 2002). This alteration in membrane stability facilitates the uncontrolled leakage of intracellular constituents, including ions and metabolic intermediates, ultimately compromising cell viability (Lambert *et al*., 2001). Beyond its effects on membrane integrity, thymol has also been shown to interfere with enzymatic systems and cellular homeostasis, thereby impairing critical metabolic pathways (Marchese *et al*., 2016). In fungal organisms, similar mechanisms have been described, with additional disruption of sterol-dependent membrane organizations contributing to growth inhibition (Marchese *et al*., 2016). The combined impact of these effects results in a progressive loss of cellular function, highlighting the broad-spectrum antimicrobial potential of thymol.

4.2 Antioxidant Mechanism

thymol exerts its antimicrobial effects primarily by targeting microbial cell membranes. Due to its lipophilic phenolic structure, thymol can readily insert into the phospholipid bilayer of bacterial and fungal membranes, altering membrane organization and increasing permeability. This disruption leads to the leakage of critical intracellular constituents, including potassium ions (K^+), protons, ATP, and other cytoplasmic molecules, ultimately collapsing the proton motive force and depleting cellular energy stores (Ultee *et al*., 1999; Lambert *et al*., 2001). Such loss of membrane integrity impairs essential processes such as respiration, nutrient uptake, and ATP synthesis, culminating in microbial cell death (Lambert *et al*., 2001).

Beyond direct membrane destabilization, thymol influences membrane-associated proteins and enzymes by modifying the lipid environment required for their optimal activity, thereby further affecting cellular metabolism (Marchese *et al*., 2016). Additionally, thymol has been reported to inhibit biofilm formation by reducing initial microbial adhesion and limiting the production of extracellular polymeric substances, which decreases microbial persistence and resistance (Nostro *et al*., 2007). Thymol also disrupts quorum sensing pathways, interfering with the regulation of virulence genes and diminishing pathogenicity in several bacterial species (Kerekes *et al*., 2013).

The antimicrobial spectrum of thymol encompasses Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus* species, Gram-negative bacteria including *Escherichia coli*, and fungi such as *Candida albicans*. In fungal organisms, thymol not only compromises membrane integrity but also perturbs ergosterol-dependent functions, contributing to its antifungal activity (Marchese et al., 2016; Ahmad *et al*., 2015).

4.3 Anti-inflammatory Mechanism

Thymol exerts its anti-inflammatory effects by modulating multiple molecular pathways involved in the inflammatory response. It has been shown to suppress the production of key pro-inflammatory cytokines, including

tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), which play central roles in initiating and amplifying inflammation (Marchese *et al*., 2016; Alavizadeh *et al*., 2020). In addition, thymol inhibits cyclooxygenase-2 (COX-2) activity, thereby decreasing prostaglandin synthesis and mitigating inflammation-associated pain, edema, and tissue injury. The compound also downregulates inducible nitric oxide synthase (iNOS) expression, limiting the excessive formation of nitric oxide (NO), a reactive mediator that contributes to oxidative stress and inflammatory tissue damage (Simoes *et al*., 2018; Ahmad *et al*., 2015). By targeting these interconnected signaling pathways, thymol effectively attenuates inflammatory responses, protects tissues from cellular injury, and demonstrates therapeutic potential in various *in vitro* and *in vivo* models, including experimental systems of arthritis, colitis, and pulmonary inflammation.

4.4 Analgesic Effects and Ion Channel Modulation

thymol, a monoterpenoid phenol present in the essential oils of *Thymus vulgaris*, oregano, and other aromatic plants, exhibits notable analgesic and antispasmodic activities through complex modulation of ion channels in both neurons and smooth muscle cells. In sensory neurons, thymol primarily affects transient receptor potential (TRP) channels, especially TRPA1 and TRPV3, which are non-selective cation channels permeable to calcium (Ca^{2+}) and sodium (Na^{+}). Low concentrations of thymol transiently activate these channels, resulting in Ca^{2+} influx and brief depolarization of the neuronal membrane; however, sustained or repeated exposure induces rapid desensitization, effectively reducing channel activity and attenuating nociceptive signaling.

Concurrently, thymol interacts with voltage-gated sodium (Nav) channels, including the Nav1.7 and Nav1.8 subtypes abundant in nociceptive fibers, by stabilizing their inactivated state. This action limits sodium entry during action potential initiation and propagation, decreases neuronal firing frequency, and reduces the release of excitatory neurotransmitters such as glutamate and substance P, thereby alleviating both peripheral and central sensitization associated with pain. In smooth muscle cells, including those of the gastrointestinal tract and airways, thymol's modulation of ion channels lowers intracellular Ca^{2+} levels, disrupting the actin–myosin contractile machinery and promoting muscle relaxation. These effects underlie thymol's capacity to reduce intestinal spasms, diminish cough reflex sensitivity, and relieve muscle cramps.

Overall, the combined modulation of TRP and Nav channels, together with downstream impacts on calcium signaling and smooth muscle tone, provides a mechanistic rationale for the traditional and experimentally validated analgesic, antispasmodic, and anti-tussive properties of thymol (Xu *et al*., 2006; Reiner *et al*., 2009; Guimarães *et al*., 2010; Huang *et al*., 2019; Akbar *et al*., 2016).

4.5 Anticancer mechanism

thymol demonstrates anticancer activity through a combination of molecular and cellular mechanisms that collectively reduce tumor cell survival, proliferation, and metastatic potential. A primary mechanism involves the induction of apoptosis via the intrinsic mitochondrial pathway. In this context, thymol disrupts mitochondrial membrane potential and activates pro-apoptotic proteins such as Bax and caspases, leading to programmed cell death in human gastric carcinoma (AGS) cells and glioblastoma U87 cells (Bajpai Sharma & Baek., 2016; Simoes *et al*., 2018). Additionally, thymol can induce cell cycle arrest, particularly at the G0/G1 phase, thereby preventing DNA synthesis and cellular division, as observed in breast cancer (MCF-7), colon cancer (HCT8, HCT116), and liver cancer (HepG2) cell lines (Racić *et al*., 2018; Gao *et al*., 2019).

Thymol also selectively increases reactive oxygen species (ROS) in cancer cells, promoting oxidative stress beyond tolerable levels and triggering apoptosis, while exerting minimal effects on normal cells (Simoes *et al*., 2018; Ahmad *et al*., 2015). Furthermore, thymol modulates key signaling pathways involved in tumor progression, including PI3K/Akt, MAPK/ERK, NF-κB, and Wnt/β-catenin, resulting in reduced proliferation, invasion, and metastasis (Al Dhabi *et al*., 2020). These effects are reinforced by the regulation of apoptotic gene expression: thymol upregulates pro-apoptotic genes such as Bax, Bak, and p53, while downregulating anti-apoptotic genes including Bcl-2, Bcl-xL, *and* XIAP (Marchese *et al*., 2016).

Supporting these mechanistic insights, El Ouahdani *et al*. (2023) reported that essential oils rich in thymol significantly decreased the viability of breast cancer (MCF-7, MDA-MB-231) and liver cancer (HepG2) cells in a dose-dependent manner. Interestingly, combinations of *Thymus algeriensis* and *Artemisia herba-alba* essential oils exhibited stronger cytotoxic effects (lower IC_{50}) than individual oils, confirming the potent anticancer potential of thymol-containing extracts. Similar observations have been reported in various *in vitro* cancer models, demonstrating thymol's ability to inhibit proliferation, induce apoptosis, and reduce migration and invasion of tumor cells (Bajpai Sharma & Baek, 2016; Gao *et al*., 2019; Racić *et al*., 2018).

Taking together, these findings indicate that thymol's anticancer activity results from a network of complementary mechanisms, including ROS-mediated apoptosis, cell cycle arrest, suppression of proliferative and metastatic signaling pathways, and modulation of apoptotic gene expression. This multi-targeted profile positions thymol as a promising candidate for further investigation as a complementary anticancer agent, although additional *in vivo* studies and clinical trials are required to confirm its therapeutic efficacy and safety (Simoes *et al*., 2018; El Ouahdani *et al*., 2023).

4.6 Gastrointestinal effects

thymol, a naturally occurring monoterpene phenol present in herbs such as *Thymus vulgaris* (thyme), has been extensively investigated for its effects on the gastrointestinal system in various experimental models. In rodent models of chemically induced colitis, oral administration of thymol consistently reduces inflammation by lowering levels of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, and decreasing myeloperoxidase activity in colonic tissues. These effects are associated with inhibition of the NF-κB

signaling pathway and enhancement of antioxidant defenses, accompanied by improvements in mucosal architecture and increased expression of barrier proteins such as occludin and ZO-1, compared with untreated controls (Zhang *et al*., 2024; Rita *et al*., 2019).

Additional studies indicate that thymol modulates the gut microbiota in colitis models, promoting the growth of beneficial *Bacteroidetes* while reducing *Proteobacteria*, which correlates with decreased inflammatory markers and restoration of short-chain fatty acid profiles supportive of mucosal health (Zhang *et al*., 2024). *In vitro* experiments using intestinal epithelial cells have further demonstrated that thymol strengthens tight junction integrity and enhances mucus secretion, mechanisms critical for maintaining barrier function and protecting against inflammatory damage (El-Sayed *et al*., 2022).

Thymol also exerts gastroprotective effects in rodent models of gastric ulceration. Pre-treatment with thymol reduces lesion formation and increases gastric mucus content, likely through modulation of endogenous prostaglandin synthesis and potassium channel activity, rather than direct inhibition of gastric acid secretion (Rozza *et al*., 2016; de Sousa *et al*., 2017). Beyond mucosal protection, thymol has been investigated for its effects on gastrointestinal motility and nausea. *In vivo* studies in chicks demonstrated that thymol decreases retching and delays the onset of emesis following an emetic challenge, while *in silico* docking studies suggest potential interactions with 5-HT3 and Dopamine D2 receptors involved in nausea and vomiting reflexes (Khan *et al*., 2023).

Preliminary research in stress-induced visceral hypersensitivity models also indicates that thymol may attenuate visceral pain and modulate serotonergic signaling within the gut-brain axis, although these findings remain largely limited to non-human models (Singh *et al*., 2020). Collectively, these studies suggest that thymol can reduce gut inflammation, enhance barrier integrity, influence microbial communities, and interact with neuro-modulatory pathways relevant to gastrointestinal function. Nevertheless, most of the evidence comes from *in vitro* and *in vivo* models, and well-designed clinical trials in humans remain scarce, limiting definitive conclusions regarding its therapeutic potential in human gastrointestinal disorders.

5. Safety and toxicity

Thymol is generally considered safe when used at low concentrations, which explains its widespread application in food flavorings, oral hygiene products, and pharmaceutical formulations. The European Food Safety Authority (EFSA) has evaluated thymol as a flavoring substance and concluded that estimated dietary intake levels do not pose safety concerns (EFSA Panel on Food Contact Materials, 2012). Its extensive history of traditional use, combined with modern toxicological assessments, further supports a favorable safety profile under conventional exposure conditions (Marchese *et al*., 2016).

However, thymol can produce adverse effects at elevated doses. Acute oral toxicity studies in rodents have reported gastrointestinal irritation, reduced locomotor activity, and central nervous system depression at high doses, with LD_{50} values indicating moderate acute toxicity depending on species and route of administration (WHO IPCS., 2002). The lipophilic phenolic nature of thymol contributes to its antimicrobial activity through membrane disruption, but this same property can lead to cytotoxicity at excessive concentrations (Marchese *et al*., 2016). Dermal contact with concentrated thymol may cause irritation or hypersensitivity reactions in susceptible individuals, and it is classified as a skin and eye irritant in high concentrations (ECHA., 2023).

Hepatotoxicity has been observed in animals exposed to high doses, as thymol is primarily metabolized in the liver via phase II conjugation pathways, including glucuronidation and sulfation, which facilitate urinary excretion (WHO IPCS., 2002). Overwhelming these metabolic pathways can lead to transient elevations in liver enzymes. Very high experimental doses have also been associated with renal changes and mild hematological alterations, although these effects occur at levels far exceeding typical dietary or oral care exposures (WHO IPCS, 2002). Data on reproductive and developmental toxicity are limited; animal studies to date do not indicate significant teratogenic effects at low to moderate doses, yet caution is advised during pregnancy and lactation due to insufficient long-term human data (EFSA., 2012).

Potential drug interactions are theoretically possible because thymol may modulate hepatic enzyme activity and membrane permeability, potentially affect the pharmacokinetics of co-administered compounds when ingest in concentrated extract form (Marchese *et al*., 2016). Nonetheless, clinically relevant interactions at dietary exposure levels have not been well documented.

Overall, thymol's safety is largely dependent on formulation, concentration, and route of administration. Food additives, mouthwashes, and topical antiseptics typically use concentrations well below toxic thresholds, providing a substantial margin of safety (FDA., 2012). For therapeutic applications involving higher doses, careful optimization and monitoring are recommended. In conclusion, thymol exhibits a favorable safety profile at conventional exposure levels recognized by regulatory authorities, with toxicity primarily associated with high or prolonged experimental doses. Further research on pharmacokinetics, chronic exposure, and potential drug interactions will help clarify its long-term safety in clinical and commercial contexts.

6. Applications and future perspectives

Thymol has attracted considerable attention across scientific and industrial fields due to its broad biological activity, natural origin, and favorable safety profile at low concentrations. Its multifunctionality underpins applications in medicine, dentistry, food preservation, agriculture, veterinary science, and pharmaceutical formulation.

In clinical and healthcare settings, thymol is widely used as an antiseptic and disinfectant. It is a key component of mouthwashes and oral care products, where its antimicrobial activity against oral pathogens and ability to reduce plaque and biofilm formation are well established (Filoche *et al*., 2005; Marchese *et al*., 2016). These effects

are largely attributed to thymol's capacity to disrupt microbial membranes and increase cell permeability (Xu *et al*., 2008; Marchese *et al*., 2016). Its combination with conventional antibiotics has shown synergistic activity, particularly against biofilm-forming and drug-resistant strains (Nazzaro *et al*., 2013; Burt., 2004).

In the pharmaceutical field, thymol is being investigated as a bioactive agent in advanced drug delivery systems. Encapsulation strategies, including nano-emulsions, liposomes, and polymeric nanoparticles, are being developed to improve their stability, bioavailability, and controlled release, addressing challenges related to volatility, hydrophobicity, and rapid metabolism (Sharifi-Rad *et al*., 2017; Rostamabadi *et al*., 2019). These formulations may enhance targeted antimicrobial and anti-inflammatory therapies by optimizing tissue penetration and sustained release.

In the food industry, thymol's antimicrobial and antioxidant properties support its use as a natural preservative, capable of inhibiting foodborne pathogens and spoilage microorganisms to extend shelf life (Burt., 2004; Hyldgaard *et al*., 2012). Active packaging incorporating thymol has also demonstrated prolonged antimicrobial effects while maintaining food quality (Prakash *et al*., 2015).

Thymol has important applications in agriculture and veterinary medicine. It functions as a biopesticide and anti-fungal agent, offering crop protection with reduced environmental impact compared to synthetic pesticides (Isman., 2000; Pavela., 2015). In beekeeping, thymol-based treatments effectively reduce *Varroa destructor* infestations, a major threat to honeybee colonies (Imdorf *et al*., 1999). In livestock, thymol-containing feed additives have been shown to improve growth performance and modulate immune responses in poultry, offering a plant-derived alternative to conventional antibiotics (Hashemipour *et al*., 2013).

Ongoing research focuses on elucidating thymol's molecular mechanisms, including interactions with cell membranes, signaling pathways, and gene expression (Xu *et al*., 2008; Marchese *et al*., 2016). Such mechanistic insight may support the rational design of thymol analogues with enhanced potency and selectivity. Combination therapies leveraging thymol's membrane-disrupting properties may further improve drug uptake and combat microbial resistance (Nazzaro *et al*., 2013). Despite its general recognition as safe at low doses, additional clinical validation and comprehensive long-term toxicological studies are needed to determine optimal therapeutic dosing and safety margins in humans. Advances in green chemistry and biotechnology may also enable sustainable production methods, including improved extraction and microbial biosynthesis pathways.

In summary, thymol's diverse pharmacological activities, natural origin, and broad-spectrum biological effects position it as a promising compound across multiple sectors. Continued interdisciplinary research integrating molecular pharmacology, formulation science, food technology, and clinical evaluation will be critical to fully realize its therapeutic and commercial potential.

7. Conclusion

Thymol is a natural monoterpenoid phenolic compound that exhibits several biological activities, notably antimicrobial, antioxidant, anti-inflammatory, analgesic, and antispasmodic effects. The available experimental data supports many of its traditional applications and underlines its therapeutic relevance. Nevertheless, additional *in vivo* studies and well-designed clinical trials are necessary to better clarify its safety, effectiveness, pharmacokinetics, and mechanisms of action in humans. Further investigation is also needed to improve its bioavailability and evaluate its long-term effects. Overall, thymol shows strong potential for future use in pharmaceutical, biomedical, and industrial applications, particularly in the development of novel natural-based therapeutic agents.

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Conflicts of Interest
The authors declare no conflicts of interest.

Data availability statement
Data will be available upon request from the corresponding author.

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