MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Bioequivalence & Bioavailability International Journal
ISSN: 2578-4803

Nanotechnology as a Tool to Improve the Biological Activity of Thymol: A Review

Mittu B[1]*, Sharma R[2], Bhat A[3], Deep S[4] and Singh M[5]

[1]National Institute of Pharmaceutical Education and Research (NIPER), India
[2]Department of Chemistry, Akal Degree College, India
[3]Department of Immunology and Molecular Medicine, Sher-e-Kashmir Insititute of Medical Sciences, India
[4]Post Graduate Government College for Girls, India
[5]DIT University, India

Review Article
Volume 7 Issue 2
Received Date: October 17, 2023
Published Date: December 08, 2023
DOI: 10.23880/beba-16000220

***Corresponding author:** Bharti Mittu, National Institute of Pharmaceutical Education and Research (NIPER), Mohal, India, Email: bharti9mittu@yahoo.com

Abstract

Thymol is a naturally occurring compound found in the essential oils of thyme, oregano, and other plants. It has been shown to have a variety of pharmacological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. In recent years, researchers have investigated the potential of thymol as an anticancer agent. However, thymol has limited solubility and poor bioavailability, which limits its use as a therapeutic agent. To overcome these limitations, researchers have developed various nano-based drug delivery systems (NDDS) to improve the efficacy of thymol. In this article, we will review the current state of research on thymol NDDS with anti-tumor activity.

Keywords: Thymol; Anticancer; Nanotechnology; Nobel Drug Delivery Systems; Natural Antioxidants

Abbreviations: BCS: Biopharmaceutics Classification System; SMEDDS: Self-Micro Emulsifying Drug Delivery System; NLCs: Nanostructured Lipid Carriers; WHO: World Health Organization; SLNs: Solid Lipid Nanoparticles; EOs: Essential Oils; EC: Ethyl Cellulose; MC: Methylcellulose.

Introduction

The oral route of medication administration is still the most popular due to its many benefits, including simplicity of administration, affordability, and improved patient compliance. The medicine must first dissolve in GI fluid following oral administration before being absorbed into the bloodstream and eventually reaching the target location [1]. Between 40% and 90% of pharmaceuticals currently under development and in use have several issues, such as low water solubility, low lipophilicity, which affects oral bioavailability, reduced enteric permeability, increased human-to-human permeability fluctuations, and lack of dose correlation [2,3]. Gastric emptying, mean residence time, GI pH and blood flow, mechanism of absorption, physicochemical factors (solubility, hydrophobicity, LogP, colligative properties), molecular factors (size, porosity, pKa, functional group), and dosage form-related factors (solid, semi-solid, and liquid) all play a role in the complex process of gastrointestinal absorption from the gut [4]. The problems can be resolved by altering the structure and chemistry of the drug, but doing so is a challenging, costly, and time-

consuming process that delays the drug's release [5]. The Biopharmaceutics Classification System (BCS) divides drugs into four classes based on their solubility and permeability after oral administration: Class I (high solubility-high permeability), Class II (low solubility-high permeability), Class III (high solubility-low permeability), and Class IV (low solubility-low permeability) [4]. Due to the numerous active components and excipients needed to obtain the necessary blood concentrations after oral administration, medications with low water solubility require high dosages, which ultimately wastes time, money, and labor [2]. Numerous types of literature address this issue and its solutions, providing a nanotechnology-based strategy for the effective development of diverse drug delivery systems to solve pharmacokinetic and therapeutic issues [3]. These include ethosomes [6], self-micro emulsifying drug delivery system (SMEDDS) [7], nanosponges [8], PLGA nanoparticles [9-11], lipospheres [2,12], hydrogels [13], microparticles [14], micelles [15], nanostructured lipid carriers (NLCs) [16], nanogels [17], microcapsules [18], nanocapsules [19]. Therefore, it is essential to use nanoformulation technologies to improve the biological effects of therapies in drug development and manufacturing processes in order to overcome the low solubility and low bioavailability [2,3]. The phytochemicals thus found possess a broad spectrum of activities that demonstrate their effectiveness in treating human diseases such as suppressing cancer cell proliferation, Alzheimer's disease, cardiovascular disease, regulating inflammatory and immune responses, microbial infection, and protecting against lipid oxidation [20,21]. Phenols comprise a collection of phytochemicals that possess low water solubility, making them a classic contender for in-depth analysis and formulation development [22]. Thymol is 4-hydroxy-3-methoxycinnamic acid, which is a maximum amount of hydroxycinnamic acids. In general, they are present in the outer layer of fruits, vegetables, roots and especially in the seed coat of cereals [23]. Most researchers classify ferulic acid as a BCS Class II drug, but there are few groups where it is reported as a BCS Class III drug [24,25]. Thymol shows a broad spectrum of activities such as antioxidant [26], anticancer agent [13], free radical scavenger [14,18], antiviral [19]. anti-inflammatory [27], hepatoprotective [28], antibacterial [29], neuroprotective [21], reduce lipid peroxidation [20] and signal transduction in biological systems [30]. Although preclinical [31], clinical studies [31,32] and FDA approval [33] demonstrate the benefits and reliability of thymol, its medical applicability is limited due to its low solubility and low bioavailability, and thus fewer therapeutics [34]. However, many researches comprehensively offer a perspective to overcome these biopharmaceutical barriers and improve their pharmacological efficacy by utilizing formulation development and nanotechnology strategies to achieve better blood solubility and bioavailability of thymol. Therefore, the rationale of this manuscript is to provide a complete overview of the synthesis, structural activity relationship, physicochemical properties, and anticancer activity of thymol. In addition, special attention was paid to various formulations and nanotechnology-based prospects for increasing the solubility, bioavailability, and pharmacological potency of thymol. We strongly believe that this review will provide the best overview of thymol drug delivery systems for formulation development to fill the preclinical and clinical gaps.

Nano-Based Drug Delivery Systems

Thymol is a potential anticancer agent that has been shown to have anti-tumor activity against various types of cancer cells, but its clinical use is limited due to its poor solubility and bioavailability. Therefore, the development of thymol NDDS may overcome these limitations and enhance its efficacy as an anticancer agent [35]. Nanoparticles are defined as particles with a diameter between one and one hundred nanometers. Nanoparticles have distinct physical and chemical properties, such as a high surface area to volume ratio, high reactivity, and the capacity to overcome biological barriers [20]. Due of these qualities, nanoparticles are prospective drug delivery devices for a variety of uses, such as cancer therapy.Drug delivery methods based on nanotechnology are now a viable option for the treatment of cancer. These methods employ nanoparticles to selectively target tumors while minimizing adverse effects on healthy organs. Nanoparticles can deliver medications specifically to cancer cells while protecting them from degradation [21]. Liposomes, polymeric nanoparticles, dendrimers, and metallic nanoparticles are a few of the nanoparticles being investigated for application in drug delivery systems [22]. Depending on the application and the medication being used, each type of nanoparticle has benefits and drawbacks. Many studies have been done on the efficiency of thymol in nanotechnology-based medication delivery systems. Polymeric nanoparticles, liposomes, and solid lipid nanoparticles have all been found to contain thymol [23]. Delivering medicines directly to the tumour location while causing the least amount of harm to healthy tissues is one of the biggest hurdles in cancer treatment. Drug delivery methods based on nanotechnology have become a potential alternative to address this issue [24]. These methods increase the concentration of the medicine in the tumour while lowering the adverse effects on healthy tissues by using nanoparticles to deliver pharmaceuticals to the tumour site [25,30].

Thymol nano-based drug delivery systems with anti-tumor activity Cancer is one of the deadliest illnesses in the world, according to the World Health Organization (WHO), and it is the second biggest cause of death, accounting for an estimated 9.6 million fatalities in 2018. Despite

advances in cancer therapy, there is a need for novel and effective therapeutic techniques [1]. One possible strategy is to employ nanotechnology to create medication delivery systems that can selectively target tumors while minimizing negative effects on healthy organs [2]. Traditional cancer therapies, such as chemotherapy and radiation therapy, include drawbacks such as damage to normal cells, drug resistance development, and partial tumor elimination [3]. As a result, alternative cancer therapy options, such as the utilization of natural chemicals like thymol, have gained traction. Despite its potential anti-tumor effects, thepoor solubility and bioavailability of thymol have hampered its practical application [1]. The useof nanoparticle-based drug delivery systems have been presented as a possible method for increasing thymol solubility and bioavailability [26]. Because of its anti-tumor characteristics, thymol, natural chemical found in thyme, has been identified as a possible candidate for use in drug delivery systems [13]. Thymol is a natural monoterpene phenol with a long history of use as a medicinal compound due to its wide range of biological activities [14]. Recent research has focused on the development of thymol-based drug delivery systems with anti-tumor activity, using nano-sized particles to improve drug delivery and efficacy. Many preclinical investigations using various cancer cell lines have assessed the anti-tumor efficacy of thymol nano-based DDS. Promising anti-tumor activity has been demonstrated by thymol nano-based DDS against breast cancer, lung cancer, liver cancer, colon cancer, and prostate cancer cells. The anti-tumor effectiveness of thymol-loaded liposomes against breast cancer cells was examined in a study by Jain, et al. The findings suggested that thymol-loaded liposomes could be used for targeted medication administration because they had a stronger anti-tumor effect than free thymol. The study also discovered that thymol-loaded liposomes caused apoptosis, a crucial mechanism for cancer cell death, in breast cancer cells [36]. Similar to this, in Rao, et al. study examined the anti-tumor effectiveness of SLNs loaded with thymol against lung cancer cells. In comparison to free thymol, the results revealed that SLNs loaded with thymol had astronger anti-tumor effect, suggesting that they could be used for targeted drug delivery [27]. The study also discovered that SLNs with thymol in them caused lung cancer cells to undergo apoptosis and cell cycle arrest. In a study published in 2020, assessed the anti-tumor effectiveness of polymeric nanoparticles loaded with thymol against liver cancer cells. The findings suggested that thymol-loaded polymeric nanoparticles could be used for targeted medication delivery because they had a stronger anti-tumor effect than free thymol [8]. The study also discovered that polymeric nanoparticles containing thymol caused liver cancer cells to undergo apoptosis. Furthermore, in a study examined the anti-tumor effects of thymol-loaded dendrimers on colon cancer cells. The findings suggested that thymol-loaded dendrimers may be used for targeted medication delivery because they had a stronger anti-tumor effect than free thymol. The study also discovered that colon cancer cells experienced cell cycle arrest and apoptosis when exposed to thymol-loaded dendrimers [37].

Thymol as an Anticancer Agent

Thymol has been shown to have anticancer activity against various types of cancer cells, including breast cancer, lung cancer, colon cancer, and leukemia [31]. The anticancer activity of thymol is thought to be mediated through multiple mechanisms, including induction of apoptosis, inhibition of cell proliferation, and modulation of signaling pathways [32]. The creation of thymol nano-based drug delivery systems with anti-tumor potential has been the focus of recent study [33]. Thymol is delivered to the tumour location using these systems using nanoparticles, such as polymeric nanoparticles or liposomes [34]. Through the usage of these nanoparticles, thymol becomes more bioavailable and can reach the tumour at larger concentrations [37]. Also, the negative effects on healthy tissues are diminished by the targeted delivery of thymol to the tumour location [38,39]. Thymol can be added to polymeric nanoparticles using several procedures, including solvent evaporation, emulsion-solvent evaporation, and nanoprecipitation [40]. These techniques rely on organic components. One such strategy is to use supercritical fluid technologies. Gases that have been compressed and heated over their critical points are known as supercritical fluids [41]. Supercritical fluids can successfully take the role of organic solvents in this situation because of their distinct advantages. The most popular supercritical fluid for making nanoparticles is carbon dioxide [42]. Drug solubility, bioavailability, and therapeutic efficacy are all goals of nano-based DDS. Thymol nano-based DDS have been created employing a variety of nanocarriers, including dendrimers, polymeric nanoparticles, solid lipid nanoparticles (SLNs), and liposomes [43,44]. Drugs that are both hydrophilic and hydrophobic can be contained within the spherical vesicles known as liposomes, which are made of a lipid bilayer. Due to their biocompatibility, low toxicity, and high drug-loading capacity, liposomes have been employed as nanocarriers for the delivery of thymol. Thymol-loaded liposomes were created and their anti-tumor effectiveness against breast cancer cells was assessed in a study by Rai M, et al. [36]. The findings suggested that thymol-loaded liposomes could be used for targeted medication administration because they had a stronger anti-tumor effect than free thymol. SLNs are solid lipid-based submicron-sized particles that can encapsulate both hydrophilic and hydrophobic pharmaceuticals. As a result of their biocompatibility, high drug-loading capacity, and prolonged drug release, SLNs have been employed as nanocarriers for the delivery of thymol. Thymol-loaded SLNs were created and their anti-tumor effectiveness against lung cancer cells was assessed

in a study by Rao, et al. In comparison to free thymol, the results revealed that SLNs loaded with thymol had a stronger anti-tumor effect, suggesting that they could be used for targeted drug delivery. Polymeric nanoparticles, which may contain both hydrophilic and hydrophobic medicines, are submicron-sized particles made of biodegradable polymers. Due to their biocompatibility, high drug-loading capacity, and prolonged drug release, polymeric nanoparticles have been employed as nanocarriers for the administration of thymol. In a work by researchers created polymeric nanoparticles that were loaded with thymol and assessed their anti-tumor effectiveness against liver cancer cells [8]. Compared to free thymol, the results showed that thymol-loaded polymeric nanoparticles had a stronger anti-tumor effect, showing their potential for targeted drug delivery. Highly branching, spherical macromolecules known as dendrimers are capable of encasing both hydrophilic and hydrophobic medicines [45]. Due to their high drug-loading capacity, low toxicity, and capacity to permeate cell membranes, dendrimers have been employed as nanocarriers for the delivery of thymol. In a 2019 study, synthesized thymol-loaded dendrimers and assessed the anti-tumor effectiveness of these molecules against colon cancer cells. The findings suggested that thymol-loaded dendrimers may be used for targeted medication delivery because they had a stronger anti-tumor effect than free thymol [37].

Mechanism of Action

Thymol nano-based drug delivery systems still lack a clear understanding of how they work. Yet, it is thought that the thymol-encapsulating nanoparticles are internalized by cancer cells via endocytosis. [46,47]. The thymol is released by the nanoparticles once they have entered the cancer cells, causing the cancer cells to undergo apoptosis. Due to their larger surface area and improved permeability, the use of nanoparticles may also promote the uptake of thymol by cancer cells. Drug delivery techniques based on thymol nanotechnology have a complicated and poorly known mechanism of action. However numerous studies have offered potential explanations for how these systems might work to inhibit tumour growth. One hypothesis is that the thymol-containing nanoparticles are taken up by cancer cells via endocytosis [48]. Nanoparticles and other extracellular material are ingested by cells by the process of endocytosis, which involves creating a vesicle around the substance and bringing it into the cell. The thymol is released by the nanoparticles once they have entered the cancer cells, where it may then work its anti-tumor actions [49]. By several various ways, thymol has been proven to cause cancer cells to undergo apoptosis, or programmed cell death [50]. Cell proliferation inhibition is one method of action. It has been demonstrated that thymol prevents cancer cells from multiplying and growing, which ultimately results in cell cycle arrest and death [51]. Also, it has been demonstrated that thymol causes cancer cells to experience oxidative stress, which in turn activates apoptotic pathways. The absorption of thymol by cancer cells may be boosted using nanoparticles in thymol administration [52]. Because of their high surface area to volume ratio, nanoparticles can interact with cancer cells more readily. Moreover, nanoparticles might make thymol more permeable across cell membranes, facilitating more effective uptake. The anti-tumor effectiveness of chemotherapeutic medications may also be improved using thymol nano-based drug delivery devices. It has been demonstrated that thymol increases the sensitivity of cancer cells to chemotherapy medications, possibly by inhibiting the activity of drug efflux pumps, which are proteins that expel pharmaceuticals from cancer cells, decreasing their efficacy [53]. Thymol might make the cell membrane more permeable, which might improve the intracellular accumulation of chemotherapeutic medicines. The type of cancer being targeted, the type of nanoparticle employed, the concentration, and the length of thymol exposure are only a few of the variables that may affect the precise mechanism of action of thymol nano-based drug delivery systems [54]. To completely comprehend the mechanism of action of these systems and to enhance their efficacy and safety, more research is required. Thymol nano-based drug delivery systems may be used to treat other disorders, like fungal infections and inflammatory ailments, in addition to their anti-tumor effect. The antifungal and anti-inflammatory characteristics of thymol have been demonstrated, and nano-based drug delivery devices may increase the effectiveness of thymol in these applications as well [55]. Overall, thymol nano-based drug delivery systems offer a promising approach to the targeted delivery of thymol for the treatment of cancer and other diseases [56]. Further research is needed to fully elucidate their mechanism of action and to optimize their efficacy and safety.A variety of malignancies, including breast, colon, and liver cancer, may be treated with thymol nano-based drug delivery systems [57]. Thymol may also be effective in treating other disorders like inflammatory diseases and fungal infections [58]. Drug delivery methods based on thymol nanoparticles may also be utilized as adjuvant therapies to increase the effectiveness of chemotherapy medications. Another study examined the use of SLNs to administer thymol for the treatment of colorectal cancer. It was published in the Journal of Drug Delivery Science and Technology. The study discovered that the thymol-loaded SLNs might cause colorectal cancer cells to undergo apoptosis, or programmed cell death, and reduces tumor growth in mice [59] (Table 1).

Drug Delivery System	Composition	Method Preparation	Mean Size (nm)	Efficiency encapsulation (%)	Result/ Concentration	Activity observed	Reference
Nanoparticles	Poly (lactic-co-glycolic acid), chitosan, albumin	Solvent evaporation	10-200	50% - 90%	0.1% -10%	Anti-tumor activity	[2,3]
Liposomes	Phosphatidylcholine, cholesterol, and dicetyl phosphate	Thin film hydration	50 -200	50% - 90%	0.1% - 5%	Anti-tumor activity	[3,4]
Microemulsions	Medium chain Triglycerides, ethyloleate, water, surfactant and co surfactant	Spontaneous emulsification method or phase inversion temperature	10-100	20% - 70%	0.1% - 5%	Anti-tumor activity	[3]
Hydrogels	Polyvinyl alcohol, Hydroxy ethyl cellulose, water or any organic solvent	Physical/chemical or enzymatic crosslinking	micrometers to millimeters	50% - 90%	0.1% - 10%	Anti-tumor activity	[3,5]

Table 1: Characteristics and anti-inflammatory activity of some drug delivery systems containing thymol.

Thymol Nano-Based Delivery Systems with Antibacterial Activity (Dr. Renu)

In the current times, it is seen that over use of antibiotics accelerates the emergence of microbial resistance and raises the prevalence of microbial infections [26]. Hence, the exploration for plant-based natural compounds exhibiting antimicrobial activity has become a key factor in preventing infections from developing antimicrobial resistance [13]. Various phytochemicals, especially the essential oils (EOs) have been extensively researched for their antibacterial action against a variety of pathogens. Some of the phenol containing essential oils such as thymol, carvacrol, citral, eugenol and cinnamaldehyde are reported to have significant antibacterial activity [14]. Since the 1960s, thymol rich essential oils have been studied for potential advantages in medical applications. The antimicrobial activities of thymol in these applications demonstrated that itexhibits significant antibacterial properties [15]. The antibacterial competence of thymol andother monoterpenes against S. aureus ATCC 68380 and E. coli ATCC 15221 isolates to damage bacterial lipid membranes for understanding their mechanisms of action was evaluated using the microdilution method. The results of the study presented the higher sensitivity of S. aureus(MIC: 0.31 mg/ml) than E. coli (MIC: 5.00 mg/ml) toward thymol [16]. In another study, theantibacterial activity of thymol against four Gram-positive bacteria including Streptococcus mutans (MTCC 890), S. aureus (MTCC 96), Bacillus subtilis (MTCC 121), S. epidermidis (MTCC 435) and one Gram-negative E. coli (MTCC 723) was evaluated by disc diffusion and microtiter plate broth dilution methods. The results revealed that thymol displayed higheractivity against S. aureus and lower activity against E. coli [17]. Al-Mariri, et al. [27] proposed thatthymol is highly effective component of Thymus SyriacusBoiss against Gram-negative bacteria such as E. coli O157, Klebsiella pneumoniae, S. typhimurium, Brucella melitensis, Yersinia enterolitica O9, Proteus spp. and P. aeruginosa (MIC ranged from <0.375 - 1.5 µl/ml) [18]. The application of thymol has been widely reported in medical, agriculture and food sectors as it is recognized as safe molecule by the U.S Food and Drug Administration [19,60].

Owing to its biodegradability, higher antimicrobial and antioxidant activity, thymol has been sought to be broadly employed in the agricultural field [60]. However, the poor physico-chemical properties including higher volatility, lower water solubility and lesser bioavailability reduces its biological activity and limits its application through aqueous medium [27,61]. Also, the higher sensitivity of lipophilic bioactive substances such as essential oils and their components including thymol towards oxygen, light and temperature, reduces their efficiency [28,29,62] and make themincapable to attain the ideal drug-able half-life period, penetration, and target drug delivery [6-8,63].

These problems might be overcome by the application of nanoparticles and biocompatible materials derived from chemical conjugation and organic reactions in order to improve drug availability, drug-ability, and bioactive drug delivery, leading to the development of safer and effective antibacterial drugs for use in the majority of prime applications [6-8,63]. Wattanasatcha, et al. [34] tested the antimicrobial activity of thymol, carvacrol, eugenol, citronellal

and terpinen-4-ol against E.coli ATCC 25922, S. aureus ATCC 25923 and *P. aeruginosa* ATCC 9027 as both free and encapsulated into a blend of ethylcellulose/methylcellulose (EC/MC) sub-micron spheres.The authors reported that thymol has the higher antibacterial activity and is found to be more effective than methyl-p-hydroxybenzoate, a usual preservative. Therefore, it was chosen for encapsulation using a polymeric shell material (EC/MC 1:1 (w/w) ratio). The encapsulated thymol particles were dispersed excellently in water and the antibacterial activity of the molecule was almost similar in free and encapsulated form. Moreover, the encapsulated thymol preservative was as effective against bacterial contamination in different cosmetic formulations (lotion, cream, and gel) as the traditional methyl paraben, even when employed at 12-52-fold lower concentrations [9].

Uncontrolled use of synthetic agrochemicals for crop protection has recently sparked severe concerns about environmental contamination and the development of resistance in phytopathogenic microorganisms. To overcome these issues, the development of bio-based/non-synthetic biocides for agriculture has emerged as a key research area. Kumari, et al. prepared an antibacterial and plant growth promoting nanoemulsion using thymol and Quillaja saponin, a glycoside surfactant of Quillaja tree. Authors reported that nanoemulsion (0.01-0.06%, v/v) prepared by a sonication method exhibited considerable in vitro growth inhibition of Xanthomonas axonopodispv. glycine of soybean (6.7-0.0 log CFU/ml) and a significant enhancements of plant growth was also recorded in plants treated with thymol nano emulsion. The authors also claimed nano encapsulated thymol as a potential antimicrobial and plant growth promoting agent for agriculture [60].

In cruciferous crops, black rot diseasecaused by Xanthomonas campestris pv. campestris (Xcc) bacterium resulted in severe yield loss globally. The excessive use of chemical pesticides to control crop diseases has elevated substantial concern over the effects on the environmental and human health. Recently, nanoparticles have attracted a lot of attention in agriculture sector due to their potential for controlling plant diseases, enhancing soil fertility and nutrient availability. So, keeping in view the merits of nanoparticles, Sreelatha, et al. [35] have prepared thymol-loaded chitosan nanoparticles (TCNPs) and tested their efficiency as antibacterial agents against Xcc in vitro by cell viability, liquid broth and live dead staining assay. TCNPs were seen to inhibit the activity of Xcc by reducing the biofilm formation and the production of xanthomonadin and exopolysaccharides. The ultrastructure reports showed the release of intracellular contents due to the membrane damage in TCNP-treated Xcc cells. The results of the study revealed TCNPs as a promising antibacterial agent against Xcc [25]. Oluoch, et al. evaluated the effectiveness of antibacterial potential of thymol and eugenol loaded chitosan nanoparticles (TCNPs and ECNPs) against Ralstonia solanacearum, the bacterial wilt-causing microbe in potatoes.

Antibacterial activities of the synthesized nanoparticles were investigated using agar dilution and colony counting methods. The encapsulation of thymol and eugenol in chitosan nanoparticles has been proven to advantageous from different perspectives, including their enhanced antibacterial activity and controlled release from the nano capsules and their protection from the surrounding medium. In comparison to the positive control, TCNP and ECNPs had increased growth inhibition percentages of 92% and 94%, respectively, demonstrating their excellent effectiveness in controlling R. solanacearum in vitro. Also, it was discovered that the MIC of encapsulated eugenol and thymol was significantly lower than that of thymol and eugenol alone.

The authors concluded that encapsulating thymol and eugenol in CNPs can prevent their deterioration and increase their antibacterial effectiveness. TCNP and ECNP therefore possess a significant potential to act as a bactericide for managing the soil borne phytopathogen [11]. Food-borne infections instigated by contaminated food pose a significant threat to community health [12]. The emergence of microbial drug resistance and consumers' preference for eco-friendly food preservatives has encouraged the innovative and hasty developments in food industry [35,64].

Owing to the significant antibacterial potential of thymol, it has been preferably employed as preservative and flavouring agent in the food industry since ancient times [20]. However, the antibacterial potential of thymol may get declined due to its higher combining tendency with lipids and insolubility in food matrix owing to its hydrophobic nature. Thus, the reduced antibacterial potential limit its application in food products. Moreover, its potent sensory properties, high degradability, and volatility, all of these will negatively impact foods sensory qualities when added in free form or at high concentrations [21-24]. To overcomethese issues, Liu, et al. [29] designed and synthesized novel antimicrobial materials by immobilization of thymol derivatives on silica particles having different morphologies (hollow mesoporous silica nanoparticles (HMSNs), MCM-41, amorphous silica). The results demonstrated that mesoporous silica nanoparticles markedly enhanced the antimicrobial activity of thymol against two representative foodborne bacteria (Staphylococcus aureus and Escherichia coli). Because of the distinctive hollow porous structure and excellent antibacterial activity, functionalized HMSNs continued to effectively suppress the growth of E. coli in apple juice. Due to the unique hollow porous structure of functionalized HMSNs, these were not only exhibited the highest antibacterial activity but also sustained the effective inhibition effect on

the growth of E. coli in apple juice. Furthermore, the analysis of the physical and chemical properties of the treated juice revealed that functionalized HMSNs scarcely have an impact on the quality of the juice [25]. Shin, et al. reported the selective antibacterial activity of Thymol-zinc oxide (TZ) nanocomposite (NC) against Staphylococcus species specially S. epidermidis. The nanocomposite was prepared by the bioconjugation of thymol (T) with ZnO nanoparticle (ZO) by employing. The cell-free supernatant of ATCC 25923 cultures was used during the production of TZ NC. The various spectroscopic techniques including XRD, FT-IR, UV–Vis were employed to characterize the product. The improved antibacterial activity and selective efficacy of TZ NC with MIC values 2-32-fold lower than thymol was observed in the current study. The proposed antibacterial mechanisms of TZ NC involve membrane rupture, suppression of biofilm formation, and modulation of new cell wall and protein-synthesis associated cellular pathways. The authors suggested that the TZ nanocomposite could enhance the selectivity and bactericidal activity of thymol against target species [30].

Thymol Nano-Based Delivery Systems with Antifungal Activity

Thymol, a natural chemical compound has gained clinical importance for their anti-septic, anti-fungal and anti-inflammatory role. The anti-fungal activity of thymol is attributed to the free-hydroxy group at C6 position of its aromatic ring structure [31]. Anti-fungal activity of thymol involves inhibition of fungal biofilm matrix (rich in polysaccharides) with increasing reactive oxygen species (ROS) concentration inside fungal cell and finally disrupting their membrane integrity (Figure 1).

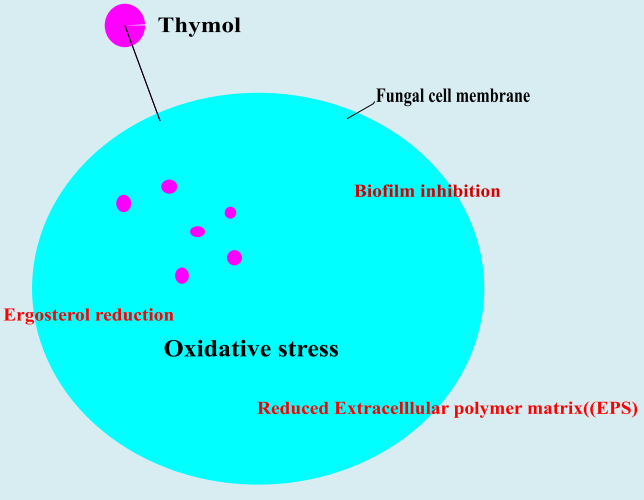

Figure 1: Thymol induced anti-fungal effect on fungal cell membrane integrity.

Biofilm formation in microbial organisms (such as fungal pathogens) is considered important virulent factor making them resistant to antimicrobial drugs [32]. Drug formulation containing thymol with hydroxyproyl methylcellulose (HPMC) and P407 (poloxamer) showed enhanced biofilm inhibitory mechanism with improved biocompatibility [33]. The anti-fungal thymol activity is found against wide variety of pathogens such as penicillium, candida tropicalis, staphylococcus aureus, cryptococcosis, aspergillus and many others. Over the past researchers by using in-vivo/in-vitro methods are developing nano-drug delivering systems using naturalcompounds such as thymol for efficient bioavailability against microbial diseases. A convectional anti-fungal treatment against oral candidiasis involving localized delivery is not efficient and requires multiple dosages. Biodegradable films carrying thymol or amphotericin B incubated with α-amylase delivery system reduced the drawbacks of conventional treatment with increased availability of anti-fungal compounds with direct contact to candida in the oral cavity [34]. Another transdermal nano-delivery method against oral candidiasis using polyacrylamide nanogel incorporating thymol enhances bioavailability and permeability with reduced development of resistant strains [37]. Nanogel preparation involves using N, N-methylene bisacrylamide with irgacure (UV cross linking initiator) loaded with thymol having particle size distribution (PDI) 0.4 and size 18.2-142.2 nm analyzed within in-vitro system could be efficient approach compared to free thymol. Nanofibrous hydrogel system incorporating essential oils (thymol, carvacrol, eugenol) with anti-fungal activities encapsulated with bacterial cellulose provides efficient double barrier delivery mechanism against fungal pathogens [38]. Limitations associated with essential oils direct use such as their high volatility, poor solubility and quick degradation are overcome with applications of nano-delivery system. Another efficient nano-drug delivery system incorporating essential oils applications involves nanoemulsion oil-based environment with approximate droplet size 20-500 nm [39]. Thymol loaded nanoparticle hydrogel (eudragit RS30D) study showed enhanced thymol retention (3.3-3.6-fold) on skin lesions formulating new wound healing therapeutic system [40]. Drug delivery system involving JNP Fe3O4 (janus magnetic nanoparticles) maximized efficacy of thymol in in-vitro study withtheir enhanced absorption inside body-fluids [18]. ThyNPMO (thymol loaded nanoparticle modified by oleic acid) drug delivery system efficacy analyzed in-vitro rat model showed protective role against olfactory cells with regulating VEGF and ROS concentrations formulating new therapeutic role against various nervous disorders such as Alzheimer, Parkinson, etc [19]. Another Nano application using thymol encapsulated within cyclodextrins (CDs) deliver system showed promising results for its future pharmaceutical applications [60]. Still further advanced research is going on

for developing advanced nano thymol drug delivery systems with reduced toxicity within animal models and enhanced bioavailability for their future pharmaceutical applications.

Thymol Nano-Based Drug Delivery Systems with Anti-Inflammatory Activity

Thymol has various medical and food packaging uses due to its exceptional pharmacological properties and anti-inflammatory, antibacterial, antifungal, and anticancer activity [41]. Many studies have been conducted on wound healing to guarantee function retention, quick healing, and less scarring [42]. The main issue with wound closure is colonization with an infectious pathogen. The skin's microbiota is beneficial in preventing the colonization of other diseases, but if it reaches a particular level at the location of a lesion, it might delay recovery [43]. Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) are the two colonizing bacteria that are most frequently shown to interfere with the initial phases of wound healing [44]. Notable effects of thymol are largely attributed to its anti-inflammatory (by preventing the release of cytokines and chemokines), antioxidant (by scavenging free radicals, enhancing endogenous enzymatic and non-enzymatic antioxidants, and chelating metal ions) and antihyperlipidemic (by raising levels of high-density lipoprotein cholesterol and lowering levels of low-density lipoprotein cholesterol in the blood) properties. Intriguingly the principal active component in thyme and oregano essential oils is thymol (5-methyl-2-isopropyphenol) [36]. Many bioactivities, including antioxidant, antispasmodic, wound-healing, and anti-inflammatory activities, have been demonstrated [45,46]. In murine macrophage cell lines, thymol (150 M) has been found to reduce LPS-induced inflammation [47]. Thymol (84 g/ml) therapy reduced in vitro macrophage inflammation brought on by lipopolysaccharide and interferon-gamma by preventing messenger RNA expression of inducible nitric oxide in J774A.1 cell lines [48]. Thymol (10 and 20 g/ml) inhibited the release of elastase, a marker of inflammatory diseases, and a serine proteinase released by activated human neutrophils when N-formyl-methionyl-leucyl-phenylalanine was applied to human polymorphonuclear neutrophils. This inhibition was concentration-dependent [49]. By inhibiting both isoforms of cyclooxygenase, thymol (100 M) has been shown to change prostaglandin catalyzed production, with cyclooxygenase -1 being the isoform most affected with an IC50 value of 0.2 M. These findings point to thymol's potential as an anti-inflammatory medication and show that it might be applied similarly to non-steroidal anti-inflammatory medicines [50]. In vitro, arachidonic acid-induced blood coagulation and platelet aggregation were inhibited by thymol (1.1 g/ml) [51]. Due to its antioxidant and anti-inflammatory characteristics, thymol (50-150 M) reduced bleomycin-induced genotoxicity in human ovarian cells (SKOV-3) [52]. By decreasing the T cell immune response and improving the T-helper cells-1 (Th1) (IL-2) and IFN-/T-helper cells-2 (Th2) (IL-4), interleukin-5, and interleukin-10 ratio in mouse primary splenocytes, Ku and Lin [53] described the anti-inflammatory properties of thymol. Thymol (40 g/ml) reduced the activity of the nuclear factor-kB (NF-B) and mitogen-activated protein kinase (MAPK) signaling pathways, which in turn reduced the inflammatory response induced by LPS in mammary epithelial cells of mouse [54].

In isoproterenol (ISO) challenged rats, an animal model of myocardial infarction (MI), which had developed myocardial necrosis, thymol (7.5 mg/kg) reversed the production of inflammation [55]. When provided at a dose of 200 mg/kg, thymol extracted from the essential oils of Lippiagracilis leaves has been demonstrated to suppress the development of carrageenan-induced paw edema in a manner comparable to that of the positive control, acetylsalicylic acid (300 mg/kg) [56]. Also, in experimental mice challenged with carrageenan, administration of this essential oil at doses of 50, 100, and 200 mg/kg prevented leukocyte migration into the peritoneal cavity. Additionally, acetic acid-induced abdominal writhes in laboratory animals were prevented by essential oil treatment.The antinociceptive and anti-inflammatory properties of Lippiagracilis are thought to be principally attributed to thymol, which was extracted from the leaf essential oils (32.68%) [57]. In the visceral pain model, it has been demonstrated to suppress the release of arachidonic acid, COX, and the formation of prostaglandins such as prostaglandin E2 (PGE2) [56]. In the acute ear edema model induced by 12-O-tetradecanoyl phorbol 13-acetate (TPA) in mice, [56] demonstrated the anti-inflammatory effect of thymol present in the Lippiasidoides essential oil administered at the doses of 1 and 10 mg/ear as evidenced by reduced edema (a 45.93 and 35.26% reduction, respectively). In various mouse models, thymol (100 mg/kg) reduced inflammation and accelerated wound healing by limiting the influx of leucocytes to the wounded areas and preventing edema [58]. By reducing the release of inflammatory mediators such as prostanoids, interleukins, and leukotrienes in the buccal locations of young adults, thymol demonstrated strong anti-inflammatory efficacy [59,65]. Thymol (10-250 g/pellet) also produced strong anti-inflammatory and antiangiogenic effects in an experiment employing the chorioallantoic membrane (CAM) of a fertilized hen's egg as the test subject [65]. In the dendritic cells isolated from the spleen of BALB/c mice, thymol (50g/ml) raised the mean fluorescence intensity (MFI) of the cluster of differentiation 40 (CD40), cluster of differentiation 86 (CD86), and major histocompatibility complex-II (MHCII) expressions [66]. Thymol significantly decreased the production of NO and H2O2 and the activities of nitric oxide synthase (NOS) and nicotinamide adenine dinucleotide reduced oxidase (NADH

oxidase) in LPS-induced murine macrophages. ROS was inhibited by thymol (IC50= 3 g/ml), and reactive nitrogen species (IC50= 4.7) [67]. According to a study of Hejazian, et al. [10], the essential oil of Carum copticum, which contains thymol as a substantial ingredient (20 mg/kg), considerably reduced pain perception in the inflammatory phase of the formalin test in mice. According to Lorente, et al. [68] when used in combination with thymol (1 mg/kg), combinations of alpha and beta- pinene (80 mg/kg) were found to potentiate the anti-inflammatory activity in female Wistar rats, In LPS-treated macrophages, thymol (10 and 20 g/ml) modulated the expression of c-Jun N-terminal kinase (JNK), stress-activated protein kinases (STAT-3), activator protein-1 (AP-1), and nuclear factors of activated T-cells (NFATs), which in turn lowered inflammatory responses [69] (Table 2).

Drug Delivery System	Composition	Method Preparation	Mean Size (nm)	Efficiency encapsulation (%)	Microorganism	Activity observed	Reference
Nanosphere	1:1 ratio of Thymol and EC/MC	Encapsulation	420	43.53	*E. coli, P. aeruginosa and S. aureus*	Antibacterial	[40]
Cationic nanoparticle	1:1 ratio of Thymol and polymer	Nanoprecipitation	36.30 to 99.41 nm	56.58 to 68.97%	*S. aureus*	Antibacterial	[40]
Alginate	Nanoparticle	Emulsification	62.54 nm	88.9% ± 1.1%	*E. coli* and *S. aureus*	Antibacterial	[41]
Alginate	Microparticle	Electrospraying	500–600 µm by	98.7% ± 0.6%	*E. coli* and *S. aureus*	Antibacterial	[41]
Chitosan grafted	Nanoparticle	Grafting	2.41-3.30 nm		*Streptococcus*	Antibacterial	[70]
Cellulose	Matrix polymer	Entrapment	1.03 ± 0.02 mm	61.20%		Antibacterial	[71]

Table 2: Characteristics and anti-inflammatory activity of some drug delivery systems containing thymol.

Conclusion

Thymol is a naturally occurring substance having a variety of biological effects, including anti-tumor effects. A promising approach to the problem of delivering medications precisely to the tumor location while limiting negative effects on healthy tissues is provided by nano-based drug delivery devices. Drug delivery methods based on thymol nanoparticles may one day be used to treat a variety of malignancies as well as other illnesses. In order to completely comprehend the mechanism of action of thymol nano-based drug delivery systems and to maximize their effectiveness and safety, additional research is required. Polymeric nanoparticles have generally shown promise as thymol drug delivery techniques, enabling greater efficacy, and overcoming some of its limitations. Nanoparticles need to have their design optimized for specific uses in order to determine their safety and biocompatibility. Yet, the potential of polymeric nanoparticles as transporters for thymol and other natural chemicals is a fascinating area of research that may lead to the development of novel therapies for a variety of diseases. In conclusion, thymol nano-based DDS have shown promising anti-tumor activity against various cancer cell lines. Thymol-loaded liposomes, SLNs, polymeric nanoparticles, and dendrimers have all been developed as nanocarriers for thymol delivery, with each having its own advantages and limitations. Thymol nano-based DDS have the potential to improve the solubility, bioavailability, and therapeutic efficacy of thymol, thereby reducing its toxicity and side effects. However, more research is needed to fully understand the pharmacokinetics and toxicity of thymol nano-based DDS in vivo. Overall, thymol nano-based DDS represent.

References

1. Li Y, Wen JM, Du CJ, Hu SM, Chen JX, et al. (2017) Thymol inhibits bladder cancer cell proliferation via inducing cell cycle arrest and apoptosis. Biochemical and Biophysical Research Communications 491(2): 530-536.

2. Assadpour E, Jafari SM (2019) A systematic review on nanoencapsulation of food bioactive ingredients and nutraceuticals by various nanocarriers. Critical Reviews in Food Science and Nutrition 59(19): 3129-3151.

3. Sampaio LA, Pina LTS, Serafini MR, Tavares DDS, Guimaraes AG (2021) Antitumor Effects of Carvacrol and Thymol: A Systematic Review. Frontiers in Pharmacology 12: 702487.

4. Rajan R, Sabnani MK, Mavinkurve V, Shmeeda H, Mansouri S, et al. (2018) Liposome-induced immunosuppression and tumor growth is mediated by macrophages and mitigated by liposome-encapsulated alendronate. Journal of Controlled Release 271: 139-148.

5. Wang B, Jeon YS, Park HS, Kim JK (2016) Self-healable mussel-mimetic nanocomposite hydrogel based on catechol-containing polyaspartamide and graphene oxide. Materials Science and Engineering C 1(69): 160-170.

6. Oluoch G, Matiru V, Mamati EG, Nyongesa M (2021) Nanoencapsulation of Thymol and Eugenol with Chitosan Nanoparticles and the Effect against Ralstonia solanacearum. Advances in Microbiology 11(12): 723-739.

7. Johny AK, Darre MJ, Donoghue AM, Donoghue DJ, Venkitanarayanan K (2010) Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on salmonella enteritidis and Campylobacter jejuni in chicken cecal contents in vitro. Journal of Applied Poultry Research 19(3): 237-244.

8. Kumari S, Kumaraswamy RV, Choudhary RC, Sharma SS, Pal A, et al. (2018) Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. Scientific Reports 8(6650).

9. Pan K, Chen H, Davidson PM, Zhong Q (2014) Thymol nanoencapsulated by sodium caseinate: Physical and antilisterial properties. Journal of Agricultural and Food Chemistry 62(7): 1649-1657.

10. Swain SS, Paidesetty SK, Padhy RN, Hussain T (2023) Nano-technology platforms to increase the antibacterial drug suitability of essential oils: A drug prospective assessment. OpenNano 9: 100115.

11. Chen H, Khemtong C, Yang X, Chang X, Gao J (2011) Nanonization strategies for poorly water-soluble drugs. Drug Discovery Today 16(7-8): 354-360.

12. Chen H, Zhang Y, Zhong Q (2014) Physical and antimicrobial properties of spray-dried zein-casein nanocapsules with co-encapsulated eugenol and thymol. Journal of Food Engineering 12(2): 337-350.

13. Huang Q, Yu H, Ru Q (2010) Bioavailability and delivery of nutraceuticals using nanotechnology. Journal of Food Science 75(1): R50-R57.

14. Meeran MFN, Jagadeesh GS, Selvaraj P (2015) Thymol attenuates altered lipid metabolism in β-adrenergic agonist induced myocardial infarcted rats by inhibiting tachycardia, altered electrocardiogram, apoptosisand cardiac hypertrophy. Journal of Functional Foods 14: 51-62.

15. Mendes SS, Bomfim RR, Jesus HCR, Alves PB, Blank AF, et al. (2010) Evaluation of the analgesic and anti-inflammatory effects of the essential oil of Lippiagracilis leaves. Journal of Ethnopharmacology 129(3): 391-397.

16. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, et al. (2015) World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. Plos Medicine 12(12): e1001923.

17. Burt S (2004) Essential oils: Their antibacterial properties and potential applications in foods - A review. International Journal of Food Microbiology 94(3): 223-253.

18. Monteiro MVB, Leite AKRM, Bertini LM, Morais SM, Pinheiro DCSN (2007) Topical anti-inflammatory, gastroprotective and antioxidant effects of the essential oil of Lippiasidoides Cham. Leaves. Journal of Ethnopharmacology 111(2): 378-382.

19. Capeletti LB, Oliveira LFD, Goncalves KDA, Oliveira JFAD, Saito A, et al. (2014) Tailored silica-antibiotic nanoparticles: Overcoming bacterial resistance with low cytotoxicity. Langmuir 30(25): 7456-7464.

20. Riella KR, Marinho RR, Santos JS, Pereira-Filho RN, Cardoso JC, et al. (2012) Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from Lippiagracilis, in rodents. Journal of Ethnopharmacology 143(2): 656-663.

21. Memar MY, Raei P, Alizadeh N, Aghdam MA, Kafil HS (2017) Carvacrol and thymol: Strong antimicrobial agents against resistant isolates. Reviews and Research in Medical Microbiology 28(2): 63-68.

22. Mahizan NA, Yang SK, Moo CL, Song AAL, Chong CM, et al. (2019) Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules 24(14): 2631.

23. Bassole IHN, Juliani HR (2012) Essential oils in combination and their antimicrobial properties. Molecules 17(4): 3989-4006.

24. Marchese A, Orhan IE, Daglia M, Barbieri R, Lorenzo AD

(2016) Antibacterial and antifungal activities of thymol: A brief review of the literature. Food Chemistry 1(210): 402-414.

25. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani, et al. (2005) Mechanisms of antibacterial action of three monoterpenes. Antimicrobial Agents and Chemotherapy 49(6): 2474-2478.

26. Mathela CS, Singh KK, Gupta VK (2010) Synthesis and in vitro antibacterial activity of thymol and carvacrol derivatives. Acta Poloniae Pharmaceutica - Drug Research 67(4): 375-380.

27. Al-Mariri A, Swied G, Oda A, Hallab LA (2013) Antibacterial activity of Thymus syriacusboiss essential oil and its components against some Syrian gram-negative bacteria isolates. Iranian Journal of Medical Sciences 38(2): 180-186.

28. Yucel-Lindberg T, Twetman S, Sköld-Larsson K, Modéer T (1999) Effect of an antibacterial dental varnish on the levels of prostanoids, leukotriene B4, and interleukin-1β in gingival crevicular fluid. Acta Odontologica Scandinavica 57(1): 23-27.

29. Liu Y, Li X, Sheng J, Lu Y, Sun H, et al. (2022) Preparation and Enhanced Antimicrobial Activity of Thymol Immobilized on Different Silica Nanoparticles with Application in Apple Juice. Coatings 12(5): 671.

30. Abdelkader A, Fathi HA, Hamad MA, Elsabahy M (2020) Nanomedicine: a new paradigm to overcome drug incompatibilities. Journal of Pharmacy and Pharmacology 72(10): 1289-1305.

31. Cimino C, Maurel OM, Musumeci T, Bonaccorso A, Drago F, et al. (2021) Essential oils: Pharmaceutical applications and encapsulation strategies into lipid-based delivery systems. Pharmaceutics 13(3): 327.

32. Singh IR, Pulikkal AK (2022) Preparation, stability and biological activity of essential oil-based nano emulsions: A comprehensive review. Open Nano 8: 100066.

33. Swain SS, Paidesetty SK, Padhy RN, Hussain T (2020) Isoniazid–phytochemical conjugation: A new approach for potent and less toxic anti-TB drug development. Chemical Biology and Drug Design 96(2): 714-730.

34. Wattanasatcha A, Rengpipat S, Wanichwecharungruang S (2012) Thymol nanospheres as an effective anti-bacterial agent. International Journal of Pharmaceutics 434(1-2): 360-365.

35. Sreelatha S, Kumar N, Yin TS, Rajani S (2022) Evaluating the Antibacterial Activity and Mode of Action of Thymol-Loaded Chitosan Nanoparticles Against Plant Bacterial Pathogen Xanthomonas campestris pv. Campestris. Frontiers in Microbiology 12: 792737.

36. Rai M, Paralikar P, Jogee P, Agarkar G, Ingle AP, et al. (2017) Synergistic antimicrobial potential of essential oils in combination with nanoparticles: Emerging trends and future perspectives. International Journal of Pharmaceutics 519(1-2): 67-78.

37. Cho TJ, Park SM, Yu H, Seo GH, Kim HW, et al. (2020) Recent advances in the application of antibacterial complexes using essential oils. Molecules 25(7): 1752.

38. Chouhan S, Sharma K, Guleria S (2017) Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. Medicines 4(3): 1-21.

39. Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology 3: 12.

40. Lorenzo JM, Khaneghah AM, Gavahian M, Marszalek K, Eş I, et al. (2019) Understanding the potential benefits of thyme and its derived products for food industry and consumer health: From extraction of value-added compounds to the evaluation of bioaccessibility, bioavailability, anti-inflammatory, and antimicrobial activities. Critical Reviews in Food Science and Nutrition 59(18): 2879-2895.

41. Naik JB, Rajput RL, Narkhede JS, Mujumdar A, Patil PB (2021) Synthesis and evaluation of UV cross-linked Poly (acrylamide) loaded thymol nanogel for antifungal application in oral candidiasis. Journal of Polymer Research 28(1): 1-13.

42. Karimi E, Abbasi S, Abbasi N (2019) Thymol polymeric nanoparticle synthesis and its effects on the toxicity of high glucose on OEC cells: Involvement of growth factors and integrin-linked kinase. Drug Design, Development and Therapy 13: 2513-2532.

43. Bose A, Sengupta P, Pal U, Senapati S, Ahsan M, et al. (2019) Encapsulation of Thymol in cyclodextrin nano-cavities: A multi spectroscopic and theoretical study. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 208: 339-348.

44. Ani EA, Heaselgrave W (2022) The Investigation of Thymol Formulations Containing Poloxamer 407 and Hydroxypropyl Methylcellulose to Inhibit Candida Biofilm Formation and Demonstrate Improved Bio-Compatibility. Pharmaceuticals 15(1): 71.

45. Ranjbar A, Ramezanian A, Shekarforoush S, Niakousari M, Eshghi S (2022) Antifungal activity of thymol against the main fungi causing pomegranate fruit rot by suppressing the activity of cell wall degrading enzymes. LWT 161: 113303.

46. Cadena KM, Arias CM, Mateo E, Urizar JMA, Quindos G, et al. (2021) In vitro activities of carvacrol, cinnamaldehyde and thymol against Candida biofilms. Biomedicine and Pharmacotherapy 143: 112218.

47. Cossu A, Wang MS, Chaudhari A, N Nitin (2015) Antifungal activity against Candida albicans of starch Pickering emulsion with thymol or amphotericin B in suspension and calcium alginate films. International Journal of Pharmaceutics 493(1-2): 233-242.

48. Mohsen AM, Nagy YI, Shehabeldine AM, Okba MM (2022) Thymol-Loaded Eudragit RS30D Cationic Nanoparticles-Based Hydrogels for Topical Application in Wounds: In Vitro and In Vivo Evaluation. Pharmaceutics 15(1): 19.

49. Shin J, Naskar A, Ko D, Kim S, Kim KS (2022) Bioconjugated Thymol-Zinc Oxide Nanocomposite as a Selective and Biocompatible Antibacterial Agent against Staphylococcus Species. International Journal of Molecular Sciences 23(12): 6770.

50. Ahmady AR, Razmjooee K, Nazar V, Samandari SS (2022) Alginate carrier as a controlled thymol delivery system: Effect of particle size. Materials Chemistry and Physics 27(2&3): 117-125.

51. Mihai MM, Dima MB, Dima B, Holban AM (2019) Nanomaterials for wound healing and infection control. Materials (Basel) 12(13): 2176.

52. Negut I, Grumezescu V, Grumezescu AM (2018) Treatment strategies for infected wounds. Molecules 23(9): 2392.

53. Mihai MM, Holban AM, Neanu CG, Popa LG, Buzea M, et al. (2014) Identification and phenotypic characterization of the most frequent bacterial etiologies in chronic skin ulcers. Romanian Journal of Morphology and Embryology 55(4): 1401-1408.

54. Okba MM, Ezzat MI, Shehabeldine AM, Ezzat SM (2023) Eurycomanol and eurycomanone as potent inducers for cell-cycle arrest and apoptosis in small and large human lung cancer cell lines. Natural Product Research 37(11): 1856-1862.

55. Salehi B, Mishra AP, Shukla I, Rad MS, Contreras MDM (2018) Thymol, thyme and other plant sources: Health and potential uses. Phytotherapy Research 32(9): 1688-1706.

56. Costa MF, Durco AO, Rabelo TK, Barreto RSS, Guimaraes AG (2019) Effects of Carvacrol, Thymol and essential oils containing such monoterpenes on wound healing: a systematic review. Journal of Pharmacy and Pharmacology 71(2): 141-155.

57. Chauhan AK, Jakhar R, Paul S, Kang SC (2014) Potentiation of macrophage activity by thymol through augmenting phagocytosis. International Immunopharmacology 18(2): 340-346.

58. Vigo E, Cepeda A, Fernandez RP, Gualillo O (2010) In-vitro anti-inflammatory effect of Eucalyptus globulus and Thymus vulgaris : nitric oxide inhibition in J774A.1 murine macrophages. Journal of Pharmacy and Pharmacology 56(2): 257-263.

59. Braga PC, Sasso MD, Culici M, Bianchi T, Bordoni L, et al. (2006) Anti-inflammatory activity of thymol: Inhibitory effect on the release of human neutrophil Elastase. Pharmacology 77(3):130-136.

60. Marsik P, Kokoska L, Landa P, Nepovim A, Soudek P, et al. (2005) In vitro inhibitory effects of thymol and quinones of Nigella sativa seeds on cyclooxygenase-1- and -2-catalyzed prostaglandin E2 biosyntheses. Planta Medica 71(8): 739-742.

61. Enomoto S, Asano R, Iwahori Y, Narui T, Okada Y, et al. (2001) Hematological studies on black cumin oil from the seeds of Nigella sativa L. Biological and Pharmaceutical Bulletin 24(3): 307-310.

62. Arab HA, Fathi M, Mortezai E, Hosseinimehr SJ (2015) Chemoprotective effect of thymol against genotoxicity induced by bleomycin in human lymphocytes. Pharmaceutical and Biomedical Research 1(1): 26-31.

63. Ku CM, Lin JY (2013) Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food Chemistry 141(2): 1104-1113.

64. Liang D, Li F, Fu Y, Cao Y, Song X, et al. (2014) Thymol inhibits LPS-stimulated inflammatory response via down-regulation of NF-κB and MAPK signaling pathways in mouse mammary epithelial cells. Inflammation 37(1): 214-222.

65. Demirci F, Paper DH, Franz G, Can Başer KH (2004) Investigation of the Origanum onites L. Essential Oil Using the Chorioallantoic Membrane (CAM) Assay. Journal of Agricultural and Food Chemistry 52(2): 251-

254.

66. Amirghofran Z, Ahmadi H, Karimi MH, Kalantar F, Gholijani N, et al. (2016) In vitro inhibitory effects of thymol and carvacrol on dendritic cell activation and function. Pharmaceutical Biology 54(7): 1125-1132.

67. Kavoosi G, da Silva JAT (2012) Inhibitory effects of Zataria multiflora essential oil and its main components on nitric oxide and hydrogen peroxide production in glucose-stimulated human monocyte. Food and Chemical Toxicology 50(9): 3079-3085.

68. Lorente I, Ocete MA, Zarzuelo A, Cabo MM, Jimenez J (1989) Bioactivity of the essential oil of bupleurum fruticosum. Journal of Natural Products 52(2): 267-272.

69. Gholijani N, Gharagozloo M, Farjadian S, Amirghofran Z (2016) Modulatory effects of thymol and carvacrol on inflammatory transcription factors in lipopolysaccharide-treated macrophages. Journal of Immunotoxicology 13(2): 157-164.

70. Chittratan P, Chalitangkoon J, Wongsariya K, Mathaweesansurn A, Detsri E, et al. (2022) New Chitosan-Grafted Thymol Coated on Gold Nanoparticles for Control of Cariogenic Bacteria in the Oral Cavit. ACS Omega 7(30): 26582-26590.

71. Karimi-Khorrami N, Radi M, Amiri S, Abedi E, McClements DJ (2022) Fabrication, characterization, and performance of antimicrobial alginate-based films containing thymol-loaded lipid nanoparticles: Comparison of nanoemulsions and nanostructured lipid carriers. International Journal of Biological Macromolecules 207: 801-812.