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Review

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Prerna Prerna , Jatin Chadha , Lavanya Khullar , Umang Mudgil , Kusum Harjai [*]

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A Comprehensive Review on the Pharmacological Prospects of Terpinen-4-ol: From Nature to Medicine and Beyond

Prerna †, Jatin Chadha †, Lavanya Khullar, Umang Mudgil and Kusum Harjai *

1. Department of Microbiology, Panjab University, Chandigarh, India
* Correspondence: kusumharjai@pu.ac.in
† Both the authors contributed equally to this work.

Abstract: Owing to their extensive biological potential, essential oils (EOs) and their bioactive phytochemicals have gained attention from the scientific community. Within this domain, Terpinen-4-ol (T-4-ol), a bioactive monoterpene alcohol and the major constituent of tea tree oil (TTO), has made its way into translational research. Recent literature on T-4-ol strongly indicates its diverse pharmacological properties, including but not limited to antimicrobial, antivirulent, antioxidant, anti-inflammatory, anti-hypertensive, and anti-cancer effects. Hence, this review provides a comprehensive overview of the multifaceted biological activities exhibited by T-4-ol, emphasizing its medicinal potential for widescale application. The antibacterial and antifungal effectiveness of T-4-ol has been discussed encompassing its role in combating a broad spectrum of bacterial and fungal pathogens, respectively. The review delves into the antivirulent prospects of T-4-ol, shedding light on its ability to attenuate virulence and mitigate bacterial pathogenesis. Scientific literature on the antioxidant and anti-inflammatory activity of T-4-ol highlighting its role in neutralizing reactive oxygen species and modulating inflammatory pathways has also been collated. Furthermore, the review elaborates on the cardioprotective and anti-hypertensive properties of T-4-ol and augments literature on its anti-cancer mechanism against various cancer cell lines. Overall, this review consolidates the existing knowledge on T-4-ol, providing a holistic understanding of its pharmacological abilities. The exploration of these diverse attributes positions T-4-ol as a promising candidate for further research and therapeutic repurposing in various biomedical applications.

Keywords: terpinen-4-ol; essential oil & bioactive phytochemical; pharmacological & biological properties; antimicrobial; anticancer; antivirulence

1. Introduction

The surge in antimicrobial resistance has been the outcome of unregulated consumption of antibiotics, resulting in a global healthcare crisis [1]. This has given rise to drug-resistant pathogenic strains, also known as superbugs. The World Health Organization (WHO) has also recognized the ESKAPE pathogens, namely *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* sp., which demand immediate attention and pose a serious threat to human health [2]. Considering the global prevalence and resistance profiles of these pathogens, present-day antibiotics are not adequate to combat bacterial infections. Hence, there is an urgent need to devise novel and efficacious antimicrobial agents to treat multidrug resistant (MDR) varieties [3]. In this regard, the scientific community has been focused on employing natural alternatives to antibiotics, such as essential oils (EOs) and bioactive compounds, which display a myriad of biological activities [4]. Due to their easy accessibility, natural existence, abundance, and economic availability, plant-derived compounds have recently drawn widespread application in mainstream medicine [5].

EOs, which are mechanically extracted from plant stems, leaves, roots, flowers, and fruits, are highly concentrated hydrophobic plant extracts enriched with a mixture of aromatically active or volatile components [6]. These include unsaturated and saturated hydrocarbons along with aldehydes, alcohols, ethers, esters, polyphenols, ketones, terpenes, and their oxygenated derivatives [6]. Among biologically renowned EOs, tea tree oil (TTO), lavender oil, eucalyptus oil, cinnamon oil, clove oil, thyme oil, lemongrass oil, and rosemary oil, have been comprehensively investigated and exploited for their pharmacological applications [7]. Phytoconstituents, also known as bioactive phytochemicals, are non-nutrient plant-derived secondary metabolites, which impart a distinct colour, aroma, and other organoleptic properties to EOs [8]. Phytochemicals have gained much popularity due to their extensive application in cosmetic and pharmaceutical industries, including aromatherapy, skin healing, permeabilization, rejuvenation, food/flavoring, and as anti-inflammatory, anti-cancer, antimicrobial agents [9]. There is a wide range of bioactive compounds known to date, out of which, alkaloids, terpenoids, phenols, phenolic glycosides, and saponins are of utmost importance [10].

Terpineols are terpenoids which are chemically modified by the addition of an alcohol group. Structurally, these are monocyclic monoterpene tertiary alcohols with four different isomeric forms, namely α-terpineol, β-terpineol, γ-terpineol, and terpinen-4-ol (T-4-ol) [11]. Their extraction naturally occurs from flowers, fruits, and spices such as apples, basil, limes, lilac, grapefruit, rosemary, eucalyptus, pine, cardamom, and cajuput oil. Among the known terpenoids, α-terpineol and T-4-ol are present in a wide variety of EOs, such as palo santo, tea tree, eucalyptus, and lavender oil, and have been widely used for their medicinal properties [11,12]. T-4-ol, also known as 4-carvomenthenol, is the principal bioactive component of TTO, constituting about 48% of the total composition, followed by γ-terpinene (28%), 1,8-cineole (15%), α-terpinene (13%), α-terpineol (8%), and p-cymene (8%). T-4-ol is also the primary phytoconstituent of thyme oil, comprising nearly one-third (33.34%) of its total composition [13]. Although essential oils contain hundreds of bioactive components, there has been a particular interest among researchers to unravel the biological properties and health benefits of T-4-ol.

T-4-ol is an isomer of terpineol with the chemical formula $C_{10}H_{18}O$ (Figure 1A). It has a 6-carbon ring, a double bond at the first carbon, making it structurally 1-menthene along with a hydroxyl substitution (alcohol functional group) at the fourth position of the parent hydrocarbon chain [14]. At room temperature, T-4-ol exists as a viscous liquid with a colourless to pale yellow appearance and a mild earthy or woody odour [15]. T-4-ol is sparingly soluble in water but is readily miscible in alcohol and oils. Under natural extraction processes, T-4-ol is isolated from its parent TTO using the vacuum fractional distillation method [16]. In a quality assessment conducted with Australian TTO, the enantiomeric range for (+)-T-4-ol was found to be 63.3-69.8% [17]. Artificially, it can be generated from sabinene through a hydration reaction or *via* photo-oxidation of terpinolene, sequentially followed by chemical reduction and selective hydrogenation of the corresponding alcohol [18]. The molecular properties of T-4-ol have been documented in Table 1. Also, computational analysis using the pkCSM server [19], which predicts the pharmacokinetic properties of a ligand, revealed that T-4-ol obeys the Lipinski's rule for druglikeliness (Table 2) and shows remarkable oral bioavailability (Figure 1B). In the past two decades, numerous investigations have confirmed diverse biological properties associated with T-4-ol, including antimicrobial, anti-quorum sensing, anti-virulence, anti-cancer, anti-hypertensive, anti-inflammatory, and antioxidant abilities (**Figure 2**). Additionally, T-4-ol has found its application as a natural disinfectant due its profound disinfection and antisepsis effects [20]. Apart from being used as a natural flavourant, T-4-ol is also incorporated in soaps, shampoos, body lotions, cleaners, and preservatives [21]. Considering its growing significance in healthcare and pharmaceutical industries, this review is the first to collate recent literature on T-4-ol, illustrating/advocating its multifaceted nature as a potent phytochemical.

(A)   (B)
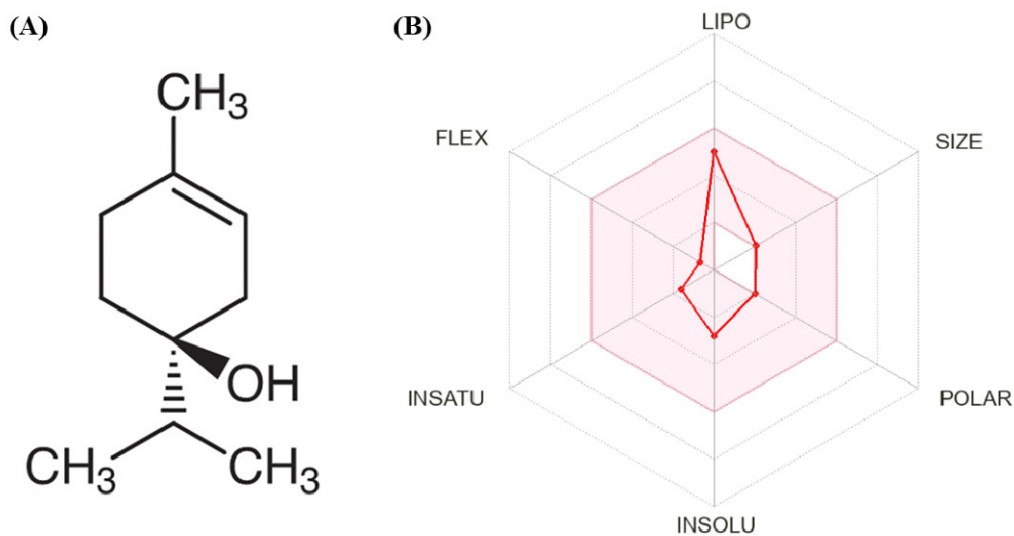
Figure 1

Figure 1. Molecular structure (A) and bioavailability radar (B) of T-4-ol [19].
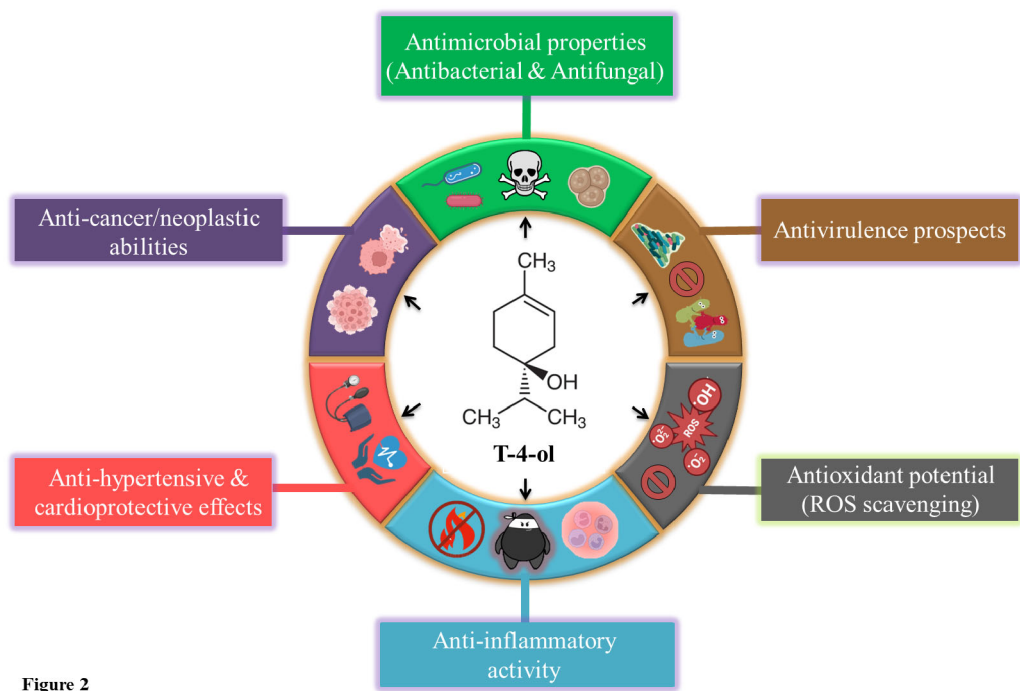
Figure 2

Figure 2. Schematic representation of various pharmacological properties of T-4-ol.

Table 1. Molecular properties of T-4-ol.

S. No.	Descriptor	T-4-ol
1	Molecular weight	154.253 g/mol
2	LogP	2.5037
3	# Rotatable bonds	1
4	# Acceptors	1
5	# Donors	1
6	Surface area	69.123

Table 2. Pharmacokinetic properties (ADMET: Absorption, Distribution, Metabolism, Excretion, Toxicity) of T-4-ol.

Pharmacokinetic property	Model name	Predicted value	Measurement units
Absorption	Water solubility	-2.386	Numeric (log mol/L)
	Caco2 permeability	1.353	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	96.364	Numeric (% Absorbed)
	Skin Permeability	-2.012	Numeric (log K_P)
	P-glycoprotein substrate	Yes	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution	VDss (human)	0.331	Numeric (log L/kg)
	Fraction unbound (human)	0.458	Numeric (Fu)
	BBB permeability	0.359	Numeric (log BB)
	CNS permeability	-2.099	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	No	Categorical (Yes/No)
	CYP1A2 inhibitor	Yes	Categorical (Yes/No)
	CYP2C19 inhibitor	No	Categorical (Yes/No)
	CYP2C9 inhibitor	No	Categorical (Yes/No)
	CYP2D6 inhibitor	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	Categorical (Yes/No)
Excretion	Total Clearance	1.056	Numeric (log mL/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
	Maximum tolerated dose (human)	1.273	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	No	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.131	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	1.872	Numeric (log mg/kg-bw/day)
	Hepatotoxicity	No	Categorical (Yes/No)
	Skin Sensitization	Yes	Categorical (Yes/No)
	T. Pyriformis toxicity	-0.009	Numeric (log μg/L)
	Minnow toxicity	1.417	Numeric (log mM)

2. Biological Properties of T-4-ol: A Comprehensive Overview

2.1. Antibacterial Potential of T-4-ol: Prospecting Alternatives to Antibiotics

The most widely explored biological aspect of T-4-ol is its remarkable antibacterial activity against Gram-positive and -negative bacterial pathogens. For the very first time, Cha *et al*. defined the chemical composition of *Cryptomeria japonica* essential oil, confirming the presence of T-4-ol (9.77%) alongside demonstrating its antibacterial effects against oral bacteria such as *Actinobacillus actinomycetemcomitans, Streptococcus mutans, Fusobacterium nucleatum, Prevotella intermedia*, and *Porphyromonas gingivalis* [22]. On similar lines, Mondello and colleagues also attributed T-4-ol (42.35%) for imparting antibacterial activity to TTO against *Legionella pneumophila* [23]. Since then, there have been numerous studies illustrating the antibacterial prospects of T-4-ol against *S. aureus*, its methicillin-resistant strains (MRSA), *P. aeruginosa, S. agalactiae, S. mutans*, and *Legionella pneumophila*. Against *S. aureus*, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of T-4-ol were found to be 0.25% and 0.5% (v/v) respectively, which corresponds to a MIC/MBC ratio of 2, signifying its bactericidal activity [24]. Further, T-4-ol has been shown to exhibit synergy with antibiotics like meropenem, oxacillin, and cefazolin, effectively inhibiting and eradicating staphylococcal biofilms [25]. The phytochemical was also

computationally predicted to interfere with cell wall biosynthesis in *S. aureus* by forming strong associations with the amino acid residues of penicillin-binding protein 2a (PBP2a) [25]. The antibacterial and antifouling efficacy of T-4-ol was also validated by Cheng *et al.* against MRSA strains using untargeted metabolomic and transcriptomic analysis [26]. Interestingly, treatment with T-4-ol (0.08%) for two hours strongly inhibited bacterial DNA and RNA biosynthesis by altering the gene expression and metabolic profile associated with purine and pyrimidine metabolism. The transcriptome profiling revealed altered expression of 16 nucleic acid synthesis genes, including *arcC*, *carB*, *deoD*, *pyrF*, *pyrB*, and *nrdF*, while metabolome analysis confirmed the reduction of 11 metabolites associated with the same pathway (2′-deoxyadenosine, cytosine, deoxyadenosine, inosine, thymidine, xanthine) [26].

The antibacterial potential of T-4-ol in combination with α-terpineol has also been recently elucidated in two different studies. T-4-ol was independently shown to harbor antibacterial properties against MRSA, *E. coli*, and *P. aeruginosa* alongside demonstrating synergy with α-terpineol [27]. Moreover, the combination of T-4-ol and α-terpineol (termed as Synterpicine™ by the authors) demonstrated excellent bactericidal activity against ESKAPE pathogens with MBC values ranging between 0.31-2.5% (v/v). Analogously, a follow-up study also confirmed the antibacterial potency and synergy of the α-terpineol-T-4-ol combination against *P. aeruginosa*, asserting the plausible application of plant bioactives to combat drug resistance [28]. Further, Zheng *et al.* demonstrated the dose-dependent antibacterial activity of T-4-ol against *S. agalactiae* through time-kill curves [29]. Apart from damaging the bacterial cell wall, T-4-ol also induced plasmolysis and increased cell membrane permeability, which was confirmed by the extracellular release of divalent cations (Ca^{2+}/Mg^{2+}) and lactate dehydrogenase. Using SDS-PAGE and DAPI staining, T-4-ol was also ascertained to interfere with protein and DNA biosynthesis in *S. agalactiae* [29]. Additionally, T-4-ol has been innovatively examined for its antibacterial potential against *L. pneumophila* using the micro-atmosphere diffusion method [30]. In its liquid as well as vapor phase, the antibacterial effectiveness of T-4-ol was found to be superior to its essential oil by nearly 2 folds up to 7 days. Moreover, T-4-ol exposure resulted in 100% bacterial killing within 10 min of phytochemical exposure, which coincided with altered cell structure and morphology, inducing swelling, wrinkling, and blebbing of bacterial cells [30]. Attempts have also been made to formulate antimicrobial micellar solutions of T-4-ol in conjunction with surfactin, cetyltrimethylammonium bromide, and cetylpyridinium chloride, for controlling oral pathogens [31]. The novel T-4-ol-containing formulation exhibited enhanced antibacterial and anti-adhesion effects against *S. mutans*, the causative agent of dental caries [31]. Another study targeting the oral pathogens, *S. mutans* and *Lactobacillus acidophilus*, highlighted the antibacterial activity of T-4-ol and its therapeutic potential in resolving dental caries [32]. Following 15 min exposure to T-4-ol at 0.24%, the expression of adhesion and biofilm-related genes in *S. mutans* (*gbpA*) and *L. acidophilus* (*slpA*) was significantly downregulated, pointing towards the antifouling properties of T-4-ol. This terpenoid phytochemical has also been shown to inhibit single- and multi-species biofilms of various periodontal pathogens at biocompatible drug concentrations [33]. Overall, these investigations provide critical evidence for the application of T-4-ol as an effective broad-range antibacterial agent.

2.2. Anti-Fungal Prospects of T-4-ol: Beyond the Antibacterial Spectrum

In addition to its antibacterial property, T-4-ol has also been explored for its antifungal activity primarily against *Candida* sp. In search of alternative therapeutics to combat drug resistance in *Candida albicans*, Mondello *et al.* comprehensively scrutinized the antifungal prospects of T-4-ol [34]. The MIC values of T-4-ol against azole-susceptible and -resistant strains of *C. albicans* ranged between 0.015-0.06% (v/v), indicating high sensitivity of fungal strains towards the terpenoid, as compared to TTO (MIC range: 0.25-0.5%). Subsequently, investigators also validated the *in vitro* findings by testing the clearance of *C. albicans* in a rat vaginal infection model *in vivo* [34]. Interestingly, intravaginal administration of T-4-ol (1%) significantly accelerated bacterial clearance from the murine vaginal tract and was deemed effective over TTO (5%). Recent investigations aimed at exploring alternate treatment strategies against oral candidiasis have also found T-4-ol as a potent

antifungal agent. One of the pre-clinical investigations indicated the MIC and minimum fungicidal concentration (MFC) of T-4-ol against *C. albicans* to be 4.5 mg/mL and 8.8 mg/mL, respectively [35]. Additionally, a brief 60 second exposure to T-4-ol at 8.8 mg/mL (oral-rinse simulation) effectively inhibited biofilm formation by *C. albicans*, as compared to TTO at 17.9 mg/mL. Another study established the synergistic interaction between T-4-ol and nystatin (antifungal) in preventing biofilm formation by *C. albicans* [36]. The authors eventually developed a liquid crystalline system containing propoxylated/ethoxylated cetyl alcohol (40%), oleic acid (40%), and chitosan (0.5%) along with T-4-ol and nystatin for combating oral candidiasis. Upon mixing artificial saliva, the novel formulation displayed increased mucoadhesion, thereby increasing the time of contact between the antifungal agents and *C. albicans*, thereby abrogating biofilm formation [36]. Besides, TTO and its bioactive phytochemicals (1,8-cineole, α-terpineol, T-4-ol, terpinolene) have also been evaluated for their antifungal activity against *Botrytis cinerea*, the causative agent of grey mould disease [37]. Among all tested phytoconstituents, the antifungal activity of T-4-ol was found to be the highest, stimulating notable alterations in cellular ultrastructure, mycelial growth, and membrane permeability of *B. cinerea* [37]. Similar effects have been noted against *Aspergillus flavus*, where T-4-ol isolated from *Pistacia lentiscus* (mastic tree) essential oil, completely inhibited mycelial growth [38]. T-4-ol has been reported for its antifungal properties against *Coccidioides posadasii* (MIC 350 µg/mL) along with yeast-like and mycelial forms of *Histoplasma capsulatum* with MIC values of 40 and 20 µg/mL, respectively [39]. Moreover, T-4-ol also results in complete killing of *Fusarium cerealis* mycelia at 0.5 % [40]. Considering all these reports documenting the broad-range antibacterial and antifungal potential of T-4-ol, it can be summarized that this phytochemical harbor potent antimicrobial activity.

2.3. Quorum Quenching and Antivirulent Potential of T-4-ol: A Recent Insight

In the post-antibiotic era, antivirulence strategies are becoming refractory to the application of antimicrobial therapies [4]. In this context, T-4-ol has been a recent addition to the list of antivirulence phytochemicals being employed against bacterial pathogens. A preliminary investigation pioneered by Kerekes and colleagues demonstrated the ability of T-4-ol to inhibit biofilm formation and violacein production, a quorum sensing (QS)-regulated bacterial pigment, in *Chromobacterium violaceum* [41]. Nevertheless, the antivirulence potential of T-4-ol was harnessed for the first time when this phytochemical was reported to interfere with the QS mechanisms of *P. aeruginosa*, thereby dysregulating virulence pathways [42]. The authors reported a synergistic interaction between T-4-ol and ciprofloxacin, which at sub-inhibitory concentrations silenced the genotypic and phenotypic expression of pseudomonal virulence factors, including pyocyanin, hemolysin, alginate, protease, and elastase production [42]. Bacterial motility phenotypes and biofilm formation in *P. aeruginosa* were also remarkably compromised upon treatment with a T-4-ol-ciprofloxacin combination. Interestingly, the QS inhibition was attributed to high-affinity interactions between T-4-ol and the QS receptors (LasR, RhlR, and PqsR) of *P. aeruginosa* [42]. In the same direction, a recent study revealed that synergistic interaction between T-4-ol and α-terpineol effectively stimulates an antivirulence response in *P. aeruginosa*, disarming phenotypic bacterial virulence through disruption of QS mechanisms [28]. Further, the anti-QS and antivirulence potential of T-4-ol have also been validated by two independent investigations using *C. violaceum*, *P. aeruginosa*, and *S. aureus* as bacterial model systems [43,44]. Contrarily, T-4-ol has been shown to abrogate biofilm formation and virulence phenotype in *B. cereus* through the upregulation of interspecies QS signals [45]. Although T-4-ol could effectively retard bacterial swarming motility, protease activity, and lower biofilm exopolysaccharides, it could not extend inhibitory effects over autoinducer-2 signals (QS molecule) in *B. cereus*. Intriguingly, the pathogen produced distinct QS signals, namely diffusing signal factors (DSFs) and diketopiperazines (DKPs), which repressed biofilm production in *B. cereus* [45]. Although these bench-based studies provide new insights into the biological prospects of T-4-ol, further mechanistic investigations are needed to validate its antivirulence potential against bacterial pathogens *in vivo*.

2.4. Antioxidant Activity of T-4-ol: On the Hunt for Radical Scavenging

Despite the versatile nature and diverse pharmacological properties of T-4-ol, its antioxidant activity has been relatively less explored. There are very few reports that document the antioxidant and radical-scavenging potential of T-4-ol. In this context, Kim *et al.* demonstrated the antioxidant activity of TTO, which was majorly attributed to three terpenic compounds, namely α-terpinene, α-terpinolene, and γ-terpinene [46]. Additionally, the authors reported that T-4-ol harbors weak antioxidant ability. Nevertheless, a plethora of contrasting studies have highlighted the antioxidant qualities of T-4-ol under oxidative stress conditions, effectively neutralizing free radicals. A recent study revealed that the administration of T-4-ol in a murine arthritis model displayed a dose-dependent antioxidant effect, which was found to be comparable to that of piroxicam [47]. T-4-ol at 60 mg/kg body weight resulted in 73.03% antioxidant activity, while piroxicam (standard antioxidant: 10 mg/kg body weight) showed 71.04% activity. Moreover, the DPPH radical-scavenging potential of T-4-ol ($48.7 \pm 0.87\%$) was superior to butylated hydroxy anisole ($44.2 \pm 0.08\%$), which was used as a standard control for the *in vitro* experimentation [47]. The authors also speculated that T-4-ol possibly extends its antioxidant potential by reducing ferric ions and lowering the activity of superoxide dismutase. Consequently, T-4-ol was proposed as a potent antioxidant that overcomes the toxicities and side effects exerted by the administration of conventional drugs used for resolving rheumatoid arthritis [47]. Furthermore, Badr *et al.* prepared separate nanoformulations of TTO and T-4-ol and scrutinized their radical-scavenging activities in terms of EC_{50} values (effective drug concentration that inhibited radical scavenging by 50%) [48]. The findings revealed that T-4-ol nanoemulsions possessed 7.72-fold higher DPPH-scavenging potential (EC_{50} = 253.65 mg/L) as compared to TTO nanoemulsions (EC_{50} = 1952.82 mg/L). As research endeavors continue to unveil the multifaceted capabilities of T-4-ol, its role as a robust antioxidant phytochemical demands further exploration, offering a hopeful prospect in the continuous pursuit of novel and efficacious therapeutic interventions.

2.5. Anti-Inflammatory Activity of T-4-ol: The Conquest Against Swelling

Another property possessed by T-4-ol that adds to its pharmacological prospects is its notable anti-inflammatory potential. Its ability to mitigate inflammatory processes provides valuable insights into the design of more effective and targeted anti-inflammatory therapies. Koh *et al.* conducted the first experimental trials demonstrating TTO's ability to reduce histamine-induced skin inflammation [49]. Brand *et al.* postulated the ability of T-4-ol (0.013%) in mitigating inflammatory responses through suppression of superoxide radical production in human neutrophils and monocytes that were chemically (N-formyl-methionyl-leucyl-phenylalanine) and biologically (lipopolysaccharide) activated *in vitro* [50]. Several other studies have further validated the anti-inflammatory prospects of T-4-ol in various animal models *in vivo*. In this context, topically administered T-4-ol (0.03-6.0 mg/paw) has been shown to avert inflammation in carrageenan-induced hind paw edema in rats [51]. Ninomiya *et al.* investigated the immunomodulatory effects of T-4-ol in combating oral candidiasis in female mice [52]. Upon infecting the immunosuppressed mice with *C. albicans,* an inflammatory response in their tongues ensued, as evidenced by elevated myeloperoxidase (MPO) activity and macrophage inflammatory protein-2 (MIP-2) in the tongue homogenates. Following treatment with T-4-ol (40 mg/mL), the expression of inflammatory markers was significantly lowered in the murine model, which coincided with suppressed cytokine secretion (TNF-α) from macrophages induced by *C. albicans* (heat-killed) at a dose of 800 µg/mL [52]. These findings suggest the anti-inflammatory role of T-4-ol as an efficacious phytochemical against oral candidiasis. Moreover, Hart and colleagues demonstrated the potential of T-4-ol (0.125%) in significantly abrogating pro-inflammatory cytokines such as prostaglandin E$_2$, interleukin (IL)-1β, IL-8, IL-10, and TNF-α in LPS-activated human monocytes, alongside displaying no signs of toxicity [12]. On parallel lines, Nogueira *et al.* reported similar inhibitory effects on IL-1β, IL-6, and IL-10 production in LPS-induced U937 monocytic cell lines [53]. However, opposing results were reported with respect to the production of TNF-α, which remained unaffected upon treatment with T-4-ol (0.059%). The authors also

concluded that T-4-ol does not modulate NF-κB or p38 MAPK pathways for cytokine production [53], thereby negating the findings illustrated by Kim and colleagues [54]. These variations may be explained by the use of different cell lines in these two investigations.

In a recent study, Zhang *et al*. tested the anti-inflammatory activity and protective efficacy of T-4-ol using human cell lines and dextran sulfate sodium (DSS)-induced experimental colitis murine model, respectively [55]. The *in vitro* findings revealed that T-4-ol notably suppressed the expression of caspase-1 and NOD-like receptor family pyrin domain containing 3 (NLRP3), thereby preventing the downstream activation of NLRP3 inflammasome in RAW 264.7 cells stimulated with LPS and lowering IL-1β secretion [55]. Further, the authors confirmed these findings *in vivo* in C57BL/6 mice by demonstrating the ability of T-4-ol in lowering MPO activity and pro-inflammatory modulators, successfully mitigating colonic damage, shortening the colon length, and ultimately the disease activity index. T-4-ol also upregulated the expression of occluding and zonula occludens-1, which got downregulated upon treatment with DSS, hence rescuing the colon epithelium barrier [55]. The molecular mechanism responsible for the anti-inflammatory effect of T-4-ol was also unraveled in NLRP3$^{-/-}$ mice, where T-4-ol administration failed to prevent DSS-induced intestinal inflammation, while the same was resolved in wild-type C57BL/6 mice (NLRP3$^{+/+}$). The study strongly suggested that T-4-ol prevents ulcerative colitis by overcoming the activation of NLRP3 inflammasome in the colon [55]. Additionally, the anti-oxidant activity of T-4-ol further contributes to its potential as a valuable anti-inflammatory agent. Reports suggest that T-4-ol could be a promising treatment option for rheumatoid arthritis by downregulating the expression levels of pro-inflammatory cytokines [47]. In the *in vivo* study, the authors developed murine arthritis models using two different chemical approaches. In the formaldehyde-induced arthritis model, subcutaneous administration of T-4-ol (15-60 mg/kg body weight) in rats significantly reduced NF-κB and TNF-α levels and downregulated IRAK mRNA expression [47]. Moreover, a remarkable increase in the levels of anti-inflammatory cytokines (IL-10 and IL-17) was observed along with T-4-ol (60 mg/kg body weight) maximally suppressing bone erosion, joint spacing, pannus formation, and cellular localization in synovial space. Contrarily, the complete Freund's adjuvant (CFA)-induced arthritis model was investigated in greater detail by examining the biochemical markers, histopathological parameters, and body weight [47]. Subcutaneous administration of T-4-ol (60 mg/kg body weight) lowered paw volume and paw thickness by 73.42% and 69.94%, respectively. The transcript levels of pro-inflammatory cytokines, including IRAK, NF-κB, TNF-α, and IL-1β, were found to be significantly reduced, while anti-inflammatory cytokines, IL-10 and IL-17, were notably elevated upon T-4-ol exposure. Furthermore, rats undergoing therapy with T-4-ol did not show any histopathological changes, exhibiting minimal synovial hyperplasia and normal hyaline cartilage, without extending any significant changes in body weight till 28 days [47]. Recently, T-4-ol has also been shown to lower inflammation and confer protection against acute lung injury in BALB/c mice [56]. Upon intraperitoneal administration of T-4-ol (5-20 mg/kg body weight) in LPS-induced acute pulmonary damage, the levels of TNF-α, IL-1β, and MPO activity were substantially reduced alongside diminishing the histopathological alterations such as lung edema, alveolar damage, and neutrophil infiltration in LPS-stimulated lung sections. T-4-ol also decreased the lung wet-to-dry weight (W/D) ratio in a dose-dependent manner. Moreover, T-4-ol alleviated the phosphorylation of NF-κB p65 and IκBα, simultaneously upregulating the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) and ultimately impeding LPS-mediated activation of NF-κB and lowering inflammation [56]. Besides, T-4-ol has also found its place as an anti-inflammatory agent in some veterinary products for inhibiting pro-inflammatory cytokines and preventing mastitis [57]. Hence, the scientific literature on T-4-ol provides fertile grounds and documents its effective anti-inflammatory properties such that it may be exploited for widescale application.

2.6. Anti-Hypertensive and Cardioprotective Effects of T-4-ol: Heart of Gold

As the research explores more phytochemical-based therapies to address hypertension-related diseases, T-4-ol has made its way into the category of hypotensive compounds. In relation to

hypertension, endothelial dysfunction has been strongly associated with increased production of reactive oxygen species (ROS), mainly superoxide radicals, and reduced nitric oxide (NO) synthesis [58]. The biosynthesis of NO is modulated by an enzyme, NO synthase (NOS), that utilizes L-arginine as a precursor. Interestingly, the deployment of L-arginine structural analogs like L-nitro arginine methyl ester (L-NAME) has been shown to induce hypertension *in vivo* through direct inhibition of NOS activity and NO production [11]. In this context, Cunha *et al.* administered L-NAME (30 mg/kg body weight) for 60 days in Wistar rats to induce chronic hypertension, which coincided with the inhibition of NO synthesis [59]. Upon intragastric administration (single dose) of *Alpinia zerumbet* EO containing T-4-ol (57.35%), the mean arterial pressure dropped significantly in a dose-dependent manner with anti-hypertensive effects extending up to 60 days. Moreover, the EO resulted in concentration-dependent vasorelaxation of rat aortic rings (intact endothelium) that were pre-contracted with phenylephrine [59]. On similar lines, T-4-ol has been reported to stimulate concentration-dependent vasorelaxation in endothelial preparations of isolated rat aorta that had been pre-contracted with a depolarizing solution of K^+ and phenylephrine [60]. Moreover, intravenous administration of T-4-ol (1-10 mg/kg body weight) in conscious normotensive and deoxycorticosterone-acetate (DOCA)-salt hypertensive rats stimulated a dose-dependent response and rapidly reduced the mean aortic pressure within 20-30 seconds. In a previous study undertaken by Lahlou and colleagues, intravenous injections of T-4-ol (bolus doses: 1-10 mg/kg body weight) immediately showcased hypotensive properties by lowering mean aortic pressure [61]. Also, T-4-ol has been shown to relax intestinal smooth muscles of rabbit duodenum precontracted with 60 mM K^+, displaying myogenic effect and dependence on calcium antagonists [62].

Another study by Maia-Joca *et al.* indicated that T-4-ol selectively inhibits electromechanical pathways associated with calcium influx through voltage-operated calcium channels (in cardiomyocytes), resulting in the relaxation of vascular smooth muscles [63]. The study illustrated that T-4-ol (100–1000 μM) attenuated the contractions caused by barium ions and phenylephrine (1 μM) in a concentration-dependent manner when aortic ring preparations were placed in a medium devoid of Ca^{2+} but enriched with K^+ (80 mM), without altering the membrane resting potential. Also, T-4-ol was capable of restoring muscular contractions that were activated by BAYK-8644, a calcium channel agonist, with an IC_{50} of 454.23 μM. Furthermore, the relaxing effect of T-4-ol on smooth muscles of rat aortic ring preparation was strongly inhibited by L-NAME. The genesis of vasorelaxant action of T-4-ol was found to be mostly myogenic, but also regulated by the integrity of the vascular endothelial layer and partially *via* the cyclooxygenase pathway [63].

Apart from hypotensive properties, T-4-ol has also been investigated for its cardioprotective abilities. Very recently, T-4-ol was shown to avert vascular calcification by abolishing endoplasmic reticulum (ER) stress-induced vascular calcification *via* sirtuin 1 (SIRT1) deacetylase [64]. Using extensive *in vitro* and *in vivo* experimentation, the authors illustrated that T-4-ol prevents calcium deposition, ER stress-mediated vascular calcification, and phenotypic switching in vascular smooth muscle cells. Treatment of vascular cells with T-4-ol significantly lowered β-glycerophosphate-induced calcium deposition, upregulated SIRT1 expression, and obstructed the activation of PERK-eIF2α-ATF4 pathway [64]. Further, treatment with T-4-ol was found to enhance SIRT1 levels, which in turn inhibited the post-translational modification of PERK (interacting partner of SIRT1), thereby lowering vascular calcification induced *via* ER stress-mediated responses. On the other hand, contradictory reports have surfaced that point towards the arrhythmogenic potential of T-4-ol [65]. In this regard, Gondim *et al.* illustrated that T-4-ol enhances Ca^{2+} entrance in cardiac cells at relatively low concentrations, while decreasing Ca^{2+} influx at higher concentrations. Consequently, this induced arrhythmia in two out of five tested rats, when administered T-4-ol through the right jugular vein at 0.01 mg/kg body weight. The study also suggests that the hypotensive effects of T-4-ol may be explained by its potential to reduce cardiac output by lowering both heart rate and stroke volume [65]. Hence, T-4-ol is a subject of great relevance since appropriate dosing is necessary to confer the desired hypotensive potential, without extending off-target effects like cardiac arrhythmias. Cumulatively, these findings pave the way for a deeper understanding of

the potential of T-4-ol in mitigating cardiovascular disorders, opening new avenues for innovative approaches in the treatment of hypertension.

### 2.7. Anti-Cancer Activity of T-4-ol: Combating a Devastating Disease

Amidst the vast array of phytochemicals, T-4-ol outshines like a wonder drug harboring remarkable anti-tumorigenic potential that has signalled a paradigm shift in oncological research [66]. The first insights into the anti-cancer prospects of T-4-ol were unraveled by Calcabrini and colleagues, wherein T-4-ol exposure induced caspase-mediated apoptosis in adriamycin-resistant and -sensitive human melanoma M14 cells [67]. A recent study by Martile *et al.* highlighted the anti-tumorigenic activity of TTO and its principle bioactive, T-4-ol, for targeted combinational therapy against melanoma cells [68]. At 18.5μg/mL, T-4-ol lowered viability of A375 and M14 cells in a concentration-dependent manner by 60% and 70%, respectively, without extending any toxic effects against human immortalized fibroblast cells (BJ-hTERT). The phytochemical also displayed synergistic effects when combined with anti-melanoma drugs, trametinib and dabrafenib, thereby inducing apoptosis *via* a direct increase in poly(ADP ribose) polymerase (PARP) and caspase 3 cleavage. The pro-apoptotic effect of T-4-ol in conjunction with the drugs was confirmed by employing a caspase inhibitor (zVAD) that prevented cell death, thereby arresting M14 cells in G1 phase of the cell cycle [68]. These findings have also been reported by Bozzuto *et al.* in adriamycin-sensitive and -resistant M14 cells, where T-4-ol exposure induces cytoskeletal reorganization and anti-proliferative effects [69]. The study illustrated that T-4-ol (0.005%) targets F-actin in the cell cytoskeleton, inhibiting the bundling of actin microfilaments in stress fibers along with disrupting the perinuclear cage, and ultimately disintegrating microtubules. Subsequently, large vimentin cables were formed in M14 cells owing to a noticeable change in the architecture of intermediate filaments [69]. Several other research groups elucidated the anti-proliferative role of T-4-ol against various cancer cells. Greay *et al.* demonstrated the dose-dependent anti-cancer effect of T-4-ol against a plethora of cell lines, including AE17 murine mesothelioma, B16 murine melanoma, L929 murine fibroblast, and HF32 human fibroblast cells [70]. Interestingly, treatment with T-4-ol (0.04 %) after 48 h was able to induce necrosis (51.6 %) and apoptosis (11.2 %) in AE17 cells, while B16 cells showed a lower degree of necrosis (9.1 %) and apoptosis (5.3 %). *via* G1 cell cycle arrest [70]. Hayes *et al.* also demonstrated the broad-spectrum anti-cancer potential of T-4-ol with IC_{50} values ranging between 60-140 mg/L, against multiple human cancer cell lines, including HeLa, HepG2, MOLT-4, K-562, and CTVR-1 [71]. In a short investigation, Casalle and Andrade reported the plausible application of T-4-ol in the treatment of oral squamous cell carcinoma [72]. The IC_{50} values of T-4-ol against HaCaT (keratinocyte), SCC-25 (squamous cell carcinoma line), and HSC-3 (squamous cell carcinoma) cell lines were shown to be 0.25%, 0.5%, and 0.25%. Further, Wu *et al.* investigated the anti-tumoral prospects of T-4-ol in human non-small cell lung cancer cells and elucidated its cytotoxic effect, which arises from the mitochondrial apoptotic pathway [73]. At 0.06%, T-4-ol stimulated cell cycle arrest in the G2/M phase, while at a higher concentration (0.08%), the phytochemical inflicted noticeable morphological changes and ultimately programmed cell death in A549 and CL1-0 cells. Consequently, T-4-ol treatment led to a series of events involving the caspase-mediated cleavage of PARP, elevating the expression of Bax protein, and lowering Bcl-2 levels in A549 and CL1-0 cells, thereby indicating the involvement of mitochondria-induced apoptosis [73]. Moreover, the authors confirmed that increased levels of p53 play a critical role in directing T-4-ol-mediated apoptosis. The findings were subsequently validated in BALB/c mice where intra-tumoral injection of T-4-ol drastically inhibited the growth of subcutaneous A549 xenografts by promoting apoptosis.

On similar lines, T-4-ol extended dose-dependent cytotoxic effects with an IC_{50} value of 155 μM against human leukemic MOLT-4 cell lines [74]. The authors revealed that T-4-ol (40 μM) triggers apoptosis through the intrinsic pathway by causing cellular damage with a significant reduction in the mitochondrial transmembrane potential through the release of cytochrome c into the cytosol. Nevertheless, T-4-ol also activated the extrinsic apoptotic pathway by enhancing caspase-8 levels, stimulating the cleavage of cytosolic Bid protein, which in turn translocates to the mitochondria and

lowers Bcl-2 expression [74]. In a follow-up study, the authors further elucidated the anti-neoplastic potential of T-4-ol against human leukemic (HL-60) cells *in vitro* [75]. Treatment with T-4-ol (12 μM) resulted in the activation of the extrinsic apoptotic pathway by inciting cytochrome c release from the mitochondria, thereby inducing caspase-8-mediated cleavage of Bid protein and lowering the levels of Bcl-xl protein. Interestingly, T-4-ol also enhanced the accumulation of autophagy-related regulatory proteins, Beclin-1, ATG5, and LC3-I/II, leading to changes in cellular morphology and development of autophagosomes in HL-60 cells [75]. T-4-ol has also been documented as a potent anti-cancer agent by inhibiting cellular proliferation in gastric (AGS), colorectal (COLO320, HCT116, HT29), prostate (CL-1, DU145), and pancreatic (MIA-PACA, Panc-1, COLO357) cancer cell lines [76]. The phytochemical exhibited synergistic inhibitory effects up to 91% and 83% with existing anti-cancer drugs such as fluorouracil (0.5 μM) and oxaliplatin (0.2 μM), respectively. Additionally, T-4-ol in conjunction with cetuximab (1 μM) exerted growth inhibition of KRAS-mutated colorectal cancer cells (anti-epidermal growth factor (EGFR) therapy resistant) by 80-90%. The combination of T-4-ol (0.2%) and cetuximab (10 mg/kg body weight) was also successful in reducing tumor volume by 63% in comparison with T-4-ol alone (40%), in nude mice xenografted with DLD1 cancer cells [76]. In the same direction, Nakayama and colleagues reported the dose-dependent anti-proliferative effects of T-4-ol against HCT116 (100-10,000 μM) and RKO (1,000-10,000 μM) colorectal cell lines with IC$_{50}$ values of 381 μM and 661 μM, respectively [77]. T-4-ol induced cytotoxicity in both the colorectal cell lines by triggering apoptosis (*in vitro*) *via* enhancing the caspase-3/7 activity, increasing annexin V-positive cells along with a marked increase in cellular SOD2 and GPX1 (ROS scavengers) protein levels. Also, *in vivo* studies with HCT116 xenografted ICR-SCID mice unveiled that T-4-ol (200 mg/kg body weight) reduced tumor size without affecting body weight. The pro-apoptotic effects of T-4-ol in xenografted mice were also validated by histopathological studies [77].

Recent investigations led by Cao and colleagues have also indicated the wide-spectrum anti-cancer potential of T-4-ol in effectively curtailing pancreatic cancer [78] and glioma [79]. The authors reported that T-4-ol (1-4 μM) significantly inhibited cellular proliferation, lowered cell migration and invasion (malignant phenotype) of AsPC-1 and PANC-1 pancreatic cell lines *in vitro* along with inducing apoptosis through increased caspase 3 cleavage and reduced Bcl-2 protein levels [78]. RNA sequencing further identified Rho-associated coiled-coil-containing protein kinase 2 (ROCK2) as the potential target of T-4-ol as it lowers its cellular levels in AsPC-1 and PANC-1 cells. These results were also validated in a subcutaneous tumorigenic model (BALB/c nude mice) where intraperitoneal injection of T-4-ol (40 mg/kg body weight, every 5 days) lowered tumor growth/progression and size accompanied by decreased expression of ROCK2 and epithelial-mesenchymal transition markers (PCNA, Ki-67, N-cadherin, and vimentin) [78]. In the subsequent study, Cao *et al*. showed that the proliferation of LN229, T98, and U251 glioma cell lines is adversely affected by T-4-ol (1-4 μM) treatment [79]. Additionally, *in vivo* experimentation unveiled that T-4-ol (40 mg/kg body weight) lowered U251-induced tumor growth and weight in BALB/c mice along with lowered expression of Ki-67 and PCNA. Further, T-4-ol significantly hindered cell growth and induced iron-mediated apoptosis (ferroptosis) in LN229, T98, and U251 glioma cells. JUN was identified as the prime target of T-4-ol *via* high-throughput screening. Interestingly, T-4-ol exposure notably suppressed JUN transcription, thereby lowering GPX4 transcript levels and promoting ferroptosis. Contrarily, overexpression of JUN prevented T-4-ol-induced ferroptosis, hence confirming its role in regulating JUN/GPX4-dependent ferroptosis and ROS levels in glioma cells [79]. The signaling pathways and molecular mechanisms associated with the anti-cancer properties of T-4-ol have been illustrated in Figure 3. In summary, all these reports provide encouraging insights into the anti-cancer prospects of T-4-ol, asserting its possible application in future cancer research and treatment strategies. Hence, at the crossroads of nature and medicine, T-4-ol stands as a guardian against malignant transformations, igniting curiosity, and prompting investigations into its profound anti-cancer properties.
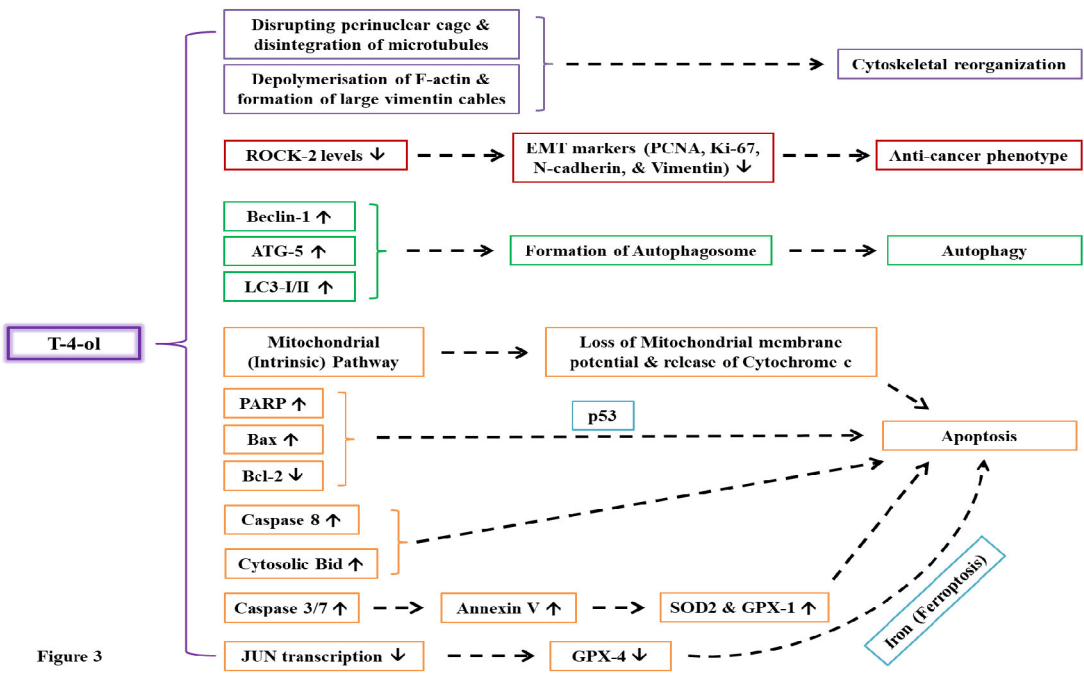
Figure 3. Molecular mechanisms underlying the anti-cancer prospects of T-4-ol. The phytochemical alters the transcription and expression of various genes associated with signaling pathways involved in apoptosis, autophagy, epithelial-mesenchymal transition, and cytoskeletal remodelling.

Apart from all the above-mentioned pharmacological properties, several other biological properties of T-4-ol have been reported in the literature. These studies have been briefly described in Table 3. Additionally, various formulations and preparations of T-4-ol have been employed for their antimicrobial, anti-fouling, photoprotective, and therapeutic applications (Table 4). These *in vitro* and *in vivo* investigations have also been successfully translated in human-based studies for their usage against multiple disease conditions in clinical settings (Table 5). In view of all the scientific evidences documented in this review, T-4-ol proves to be a formidable phytochemical harboring a wide range of pharmacological prospects.

Table 3. Other reported biological/pharmacological properties of T-4-ol.

S.No.	Pharmacological property reported	Effects described	Reference
1	Nematicidal activity	Moderate activity against adult worms of *Bursaphelenchus xylophilus* with LD_{50} 2.61 mg/mL	[80]
2	Acaricidal/Pesticidal activity	T-4-ol (2.1%) killed 85% of *Sarcoptes scabiei* (scabies mites) within 1 h and lowered worm survival rates significantly	[81]
3	Anticonvulsant activity	Administering T-4-ol (200 mg/kg body weight) decreased spontaneous motor activity within 30 min of dosing and lowered pentylenetetrazole- and picrotoxin-induced convulsions in Swiss mice	[82]
4	Anticonvulsant activity	Intraperitoneal injection of T-4-ol (0.1 and 1.0 mM) inhibited pentylenetetrazole-induced convulsions in Wistar rats and Swiss mice	[83]
5	Anti-parasitic activity	T-4-ol (1%) effectively killed *Demodex* mites within 88 min of exposure *in vitro*.	[84]

		Demodex-infested patient receiving Cliradex lid cleanser (containing T-4-ol) for 8 weeks resulted in worm clearance and clearer eyelashes	
6	Protection against inflammatory bowel disease	Treatment with T-4-ol attenuated LPS-induced damage in intestinal porcine epithelial cell lines (IPEC-J2) *in vitro* and lowered DSS-stimulated colitis in C57BL6/J mice *in vivo* by preventing LPS-mediated phosphorylation of ERK	[85]
7	Improved biomaterial quality and softness	Incorporation of cinnamaldehyde and T-4-ol to tissue conditioner (Softone™) lowers Shore A hardness of prosthodontic dentures, thereby reducing the amount of plasticizers and imparting anti-fungal potential against *C. albicans*	[86]
8	Anti-viral activity	Exposure with T-4-ol at 75 and 100 μg/mL resulted in 68.9% and 99.6% killing of herpes simplex virus-1	[87]
9	Quality indicator	Poor quality/stability of Spanish mandarin juices was assessed by the formation of ill-flavouring constituents (T-4-ol and α-terpineol) and decomposition of linalool and D-limonene	[88]
10	Anesthetic activity	T-4-ol (300-1000 μL/L) induced anesthesia in *Rhamdia quelen* (silver catfish) with induction times ranging between 103-630 s and recovery period of 134-673 s	[89]
11	Induction of disease resistance	T-4-ol treatment of post-harvest strawberry inoculated with *Botrytis cinerea* prevented disease incidence of by 44.4% after 48 h through upregulation of genes involved in phenylpropanoid biosynthesis and flavonoid metabolism pathways	[90]
12	Anti-trypanosomal activity	T-4-ol treatment (1% and 2%) resulted in 100% killing of *Trypanosoma evansi* within 3 h *in vitro* but failed to protect mice against trypanosomal infection (mastigote form)	[91]

Table 4. Studies documenting novel formulations/preparations of T-4-ol.

S. No.	Formulation reported	Effects described in the study	Reference
1	Biodegradable antifouling coating for marine applications	Deposition of T-4-ol on cover slips using a two-stage technique involving plasma-enhanced chemical vapor deposition increased surface smoothness, hydrophobicity, and transmission efficiency. T-4-ol-based coatings were also successfully field-tested for their antifouling properties in Curralea Lake, Australia	[92]
2	Plasma-polymerized thin films	T-4-ol-derived plasma polymerized films demonstrated antimicrobial and antifouling activity against *S. aureus*. Additionally, the coatings exhibited biocompatibility towards human fibroblast cells and BALB/c mouse macrophages	[93]

3	Pulse plasma-assisted thin films	Thin films deposited on borosilicate glass formed stable coatings that retained the monomeric structure of T-4-ol, increasing surface wettability (water contact angle), surface energy, and imparting antibacterial potential against *P. aeruginosa*	[94]
4	Plasma-assisted thin films	Zinc oxide nanoparticle-modified T-4-ol plasma polymers were developed and biophysically characterized. The functionalized films demonstrated improved hydrophobicity with a marked increase in the water contact angle, UV absorption, and enhanced antibacterial properties (~ 3 folds) against *E. coli*	[95]
5	Liquid-crystalline preparations for photoprotection	Biophysically characterized liquid-crystalline formulations of T-4-ol-functionalized titanium dioxide nanoparticles showcased hexagonal phase structures that permitted consistent release of T-4-ol by obeying zero-order kinetics. Controlled release of titanium dioxide nanoparticles and T-4-ol from the formulation showed photoprotective properties, indicating their possible application as a transparent inorganic sunscreen	[96]
6	Solid inclusion preparation	Novel formulation composed of solid T-4-ol/β-cyclodextrin inclusion complex prepared using freeze-drying with enhanced stability, sustained drug release, and antibacterial activity (1.25-5.0 mg/mL) against *E. coli*, *P. aeruginosa*, and *S. aureus*	[97]
7	Nanofibrous film for wound healing	Incorporating liposomal preparation of T-4-ol (6%) improved the structure and morphology of chitosan/polyethylene oxide nanofibers. The nanofibrous dressing effectively absorbed simulated tissue fluid, maintaining optimal moisture content, and mimicking human skin properties. It harbored pro-coagulant property, *in vitro* biodegradability along with antibacterial activity against *C. albicans*, *E. coli*, and *S. aureus*	[98]
8	Biodegradable nanoparticles for drug delivery	T-4-ol-loaded zein (corn protein) nanoparticles were formulated with an encapsulation efficiency of 91% for prolonged and sustained release of T-4-ol for its possible application in the treatment of melanoma	[99]
9	Nanoencapsulation for improved drug delivery	*Rhamdia quelen* (silver catfish) treated with nanoformulations of T-4-ol (5-25 mg/L for 6 days) prevented *Aeromonas hydrophila* infection and improved survival rates of silver catfish	[100]
10	Liposomal nanoparticles for antifungal prospects	Polyethylene glycol-stabilized lipid nanoparticles-containing T-4-ol displayed remarkable stability, high-capacity drug loading, sustained release of the phytochemical accompanied with antifungal and antifouling properties against *C. albicans* (MIC ~ 5 μg/mL and MBEC ~ 10 μg/mL). Moreover, the liposomal formulation delivered T-4-ol into the yeast mitochondria, thereby disrupting enzyme-dependent cellular respiration and impairing biofilm formation	[101]

Table 5. Clinical trials and investigations undertaken with T-4-ol in human subjects.

S.No.	Target disease	Study design	Number of Participants	Findings/Results	Trial number	Reference
1	Seborrheic blepharitis	Randomized, open, two-parallel group comparative investigation	48	Cleansing wipes impregnated with T-4-ol (2.5%) and hyaluronic acid (0.2%), termed 'Blephademodex®', substantially lowered ocular discomfort associated with *Demodex* (eyelash mite) blepharitis on day 8 and 29 in both the groups with no signs of allergy. Total cylindrical dandruff was reduced by 30.4% in Group 1 (once daily) and 43.5% in Group 2 patients (twice daily)	Not available	[102]
2	Seborrheic blepharitis	Randomized, double-blind, comparative clinical trial	48	Lid wipes containing hyaluronate and T-4-ol (Hy-ter®) was more efficacious over baby shampoo in reducing blepharitis symptoms at 8th and 12th weeks. Although both the treatments resulted in lowered *Demodex* count, non-invasive tear breakup time was higher for lid wipes (4 weeks) as compared to baby shampoo (8 weeks)	NCT04441528	[103]
3	Chronic blepharitis	Randomized and parallel clinical trial	17	Lid scrubs loaded with T-4-ol were parallelly assessed along placebo group in treating ocular demodicosis. Changes in *Demodox* mite count (primary objective) and changes in bulbar conjunctival hyperemia and lid margin redness (secondary objective) were monitored. Phase I trials completed but data not released in public domain	NCT01647217	[104]
4	*Demodex* blepharitis	Randomized and double-masked investigation	46	*Demodex folliculorum* infestation levels were significantly lowered with both terpinen-4-ol lid scrubs (Cliradex) and sham lid scrubs following on-site microblepharoexfoliation. However, clinical significance could not be elucidated	Not available	[105]
5	*Demodex* blepharitis	Retrospective observational case series	30	Cyclic treatment with T-4-ol-soaked wipes for 2 weeks (twice a day) was successful in improving lid margin, tear breakup time, and ocular surface disease index. Following second cycle after 7-10 gap, disease symptoms were lowered along with significantly improved tear function tests	Not available	[106]

3. Conclusion

The multifaceted biological properties of T-4-ol unfold a compelling narrative of its potential as a versatile therapeutic agent. Considering the colossal literature available on this aspect, T-4-ol warrants further scrutiny to validate its pharmacological potential in animal models such that the bench-based research may be successfully translated into clinical trials for medicinal application. Altogether, the current scientific evidences on T-4-ol incite curiosity and help in bridging the gaps between natural sources and innovative medicine for combating human diseases. The depth of its pharmacological repertoire, including the antibacterial, antifungal, antivirulent, antioxidant, anti-inflammatory, anti-hypertensive, cardioprotective, and anti-cancer properties, positions T-4-ol as a captivating subject for continued research. Thus, T-4-ol proves to be a potent phytochemical that offers promising biological prospects for its utilization in various biomedical applications and in the evolving landscape of phytochemical-based intervention strategies.

Author Contributions: **Prerna:** Data curation, Investigation, Literature survey, Writing-original draft. **Jatin Chadha:** Idea conceptualization, Investigation, Validation, Data curation, Data analysis, Supervision, Writing-original draft, review, and editing. **Lavanya Khullar:** Data curation, Validation, Data analysis,

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Writing-original draft and editing, **Umang Mudgil:** Literature survey, Formal analysis. **Kusum Harjai:** Idea conceptualization, Supervision, Validation, Continuous motivation, Writing-review and editing.

Conflict of Interest Statement: The authors declare that there is no conflict of interests.

Data Availability Statement: All the datasets generated and analyzed during the current study have all been cited in this manuscript.

Abbreviations

EO: Essential oil, IC_{50}: Inhibitory concentration (50%), IL: Interleukin, LPS: lipopolysaccharides, MIC: Minimum inhibitory concentration, MRSA: Methicillin-resistant *Staphylococcus aureus*, NF-κB: Nuclear factor kappa B, QS: Quorum sensing, ROS: Reactive oxygen species, T-4-ol: Terpinen-4-ol, TNF-α: Tumor necrosis factor-alpha, TTO: Tea tree oil.

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