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A PROMISING BIOACTIVE COMPONENT TERPINEN-4-OL: A REVIEW

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ABSTRACT: Since, the middle ages, essential oils have been widely used for bactericidal, fungicidal, insecticidal, virucidal, cosmetic and medicinal applications, especially nowadays in agriculture, food, sanitary and pharmaceutical industries. Distillation is the common mode of extraction used for aromatic plants, which results in a variety of volatile molecules such as terpenes, terpenoids, aliphatic components, and phenol derived aromatic components. Extensive research has been done in the last decade to screen and evaluate the pharmaceutical potential of the phytochemical constituent, terpinen-4-ol. It is found in various plants species and has gained attention because of its antiviral, antibacterial, anti-inflammatory and antifungal properties. This monoterpene is a bioactive component of tea tree oil (TTO) present in a plant belonging to the genus *Melaleuca*. In the present review, an overview of the current status and knowledge on the physicochemical properties, analytical techniques, formulations, pharmacological applications, irritation and toxicity potential of terpinen-4-ol have been discussed.

INTRODUCTION: At present, approximately 3000 essential oils are known, 300 of which are commercially important, especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries [1]. Major plant-derived secondary metabolites, monoterpenes are widely found in vegetables, fruits, and herbs, and play a vital role in the plant's defense mechanism. The monoterpenes consisting of two isoprene units are found abundantly in essential oils [2-3]. Additionally, many monoterpenes have displayed anticancer potential including leukemia, breast, skin, pancreatic and colon cancers in rodents [4].

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Terpinen-4-ol, one of the primary active ingredients of the TTO, consists of more than 100 different compounds. It is available in a variety of aromatic plants like New Zealand lemonwood tree, oranges, mandarins, origanum and Japanese cedar and, black pepper in leaves, flowers, and herbs[6]. Recently, it has been extracted from the leaves of *Melaleuca alternifolia* species (belonging to family Myrtaceae).

This monoterpene is also known by several other names like 4-methyl-1-propan-2-ylcyclohex-3-en-1-ol, 4-carvomenthenol; *p*-menth-1-en-4-ol; 4-terpineol and 1-terpinen-4-ol [7]. It is used in artificial pepper and geranium oil, and in perfumery for creating herbaceous and lavender notes.

It is a liquid having various properties. It gained attention due to its antibacterial, antiviral, antifungal and anti-inflammatory properties [1, 8-10].

TABLE 1: PLANTS CONTAINING TERPINEN-4-OL AS ONE OF THEIR CONSTITUENT [5]

S. no.	Genus species	Family	Part	Concentration
1	*Agathosma betulina*	Rutaceae	Leaf	-
2	*Barosma betulina*	Rutaceae	Leaf	-
3	*Camellia sinensis*	Theaceae	Leaf	-
4	*Carica papaya*	Cariaceae	Fruit	-
5	*Citrus aurantium*	Rutaceae	Plant	-
6	*Coridothymus capitatus*	Lamiaceae	Shoot	0-155 ppm
7	*Melaleuca alternifolia*	Myrtaceae	Leaf	30-48 (%)
8	*Origanum onites*	Lamiaceae	Shoot	0-525 ppm
9	*Origanum vulgare* (subsp. hirtum)	Lamiaceae	Shoot	0-90 ppm
10	*Psoralea corylifolia*	Fabaceae	Seed	-
11	*Satureja thymbra*	Lamiaceae	Shoot	0-185 ppm
12	*Thymus vularis*	Lamiaceae	Plant	8320 ppm

Physicochemical Properties: Besides active constituents present in TTO, terpinen-4-ol is its major constituent (ISO 4370 range: 30-48%) which is responsible for various activities of oil. **Fig.1**. depicts the chemical structure of this constituent [11]. It belongs to monoterpenes. It is slightly colorless to pale yellow liquid [12] with herbal pepper flavoring [13], and pine odor [14]. It is miscible with water $3.87 \times 10^{+2}$ mg/l at 25 ºC [15]. Its molecular weight is 154.24932 g/mol. It has a boiling point. 209 ºC, density 0.926 g/cu cm at 20 ºC [16] and vapour pressure: 0.04 mm Hg at 25 ºC [17]. With Log P log Kow = 3.26, it is highly lipophilic in nature [18].

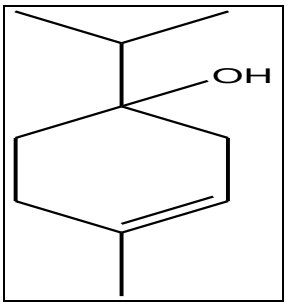

FIG. 1: CHEMICAL STRUCTURE OF TERPINEN-4-OL

Pharmacological Applications of Terpinen-4-ol:

1. Anti-inflammatory: Terpinen-4-ol was able to diminish the production of TNF-α, IL-8, IL-1β, IL-10, and prostaglandin E2 by lipopolysaccharide-activated monocytes. Terpinen-4-ol along with α-terpineol is also found to suppress superoxide production by agonist-stimulated monocytes [19]. In contrast, in similar work, it was found that TTO retards the production of reactive oxygen species by both stimulating neutrophils and monocytes, and that it also stimulates the production of reactive oxygen species by nonprimed neutrophils and monocytes [20].

These studies discovered specific TTO mechanisms which may diminish the normal inflammatory response *in-vivo*. Topically applied TTO has also been shown to modify the edema associated with the efferent phase of contact hypersensitivity in mice [21] but not the development of edema in the skin of no sensitized mice or the edematous response to UV-B exposure. This activity was primarily due to terpinen-4-ol and α-terpineol. Recently, it has been shown that terpinen-4-ol, but not 1, 8-cineole or α-terpineol, modify the plasma extravasation and vasodilation associated with histamine-induced inflammation in humans [22].

2. Anti-microbial: The inhibitory effect of *Melaleuca alternifolia* (tea tree) essential oil on the development of antibiotic resistant *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* was investigated. Frequencies of single-step antibiotic-resistant mutants were measured by inoculating bacteria cultured with or without sub-inhibitory TTO onto agar (containing 2 to 8 times the minimum inhibitory concentration (MIC) of each antibiotic). Relatively minor differences in resistance frequencies were observed in most cases, only the combination of TTO and kanamycin resulted in 10-fold fewer resistant *E. coli* mutants in comparison to kanamycin alone.

The development of antibiotic resistance in the presence of terpinen-4-ol or TTO was checked by culturing *S. aureus,* and *E. coli* isolates daily (with antibiotic alone, antibiotic with TTO) and antibiotic with terpinen-4-ol (for 6 ds). Increase in median MIC for each antibiotic alone was 4 to 16-fold. Subinhibitory terpinen-4-ol or TTO did not greatly alter results, with d 6 median MICs being either the same as or one concentration different from those

for antibiotic alone. For terpinen-4-ol and TTO alone, d 6 median MICs had enhanced 4-fold for *S. aureus* (n = 18) and 2-fold for *E. coli* (n = 18) from baseline. Lastly, few remarkable changes in antimicrobial susceptibility were observed for *S. aureus* and *S. epidermidis* isolates that had been serially subcultured 14 to 22 times with subinhibitory terpinen-4-ol. Overall, the data exhibited that terpinen-4-ol and TTO have little impact on the development of antimicrobial resistance and susceptibility [23].

3. Anti-fungal: TTO has been evaluated as a potential antifungal agent against *Botrytis cinerea (B. cinerea)*. In a study, antifungal activity and mode of action of TTO and its components against *B. cinerea* were checked. Among the components, terpinen- 4-ol showed the highest antifungal activity, followed by TTO, α-terpineol, terpinolene, and 1, 8-cineole. Terpinen-4-ol treatment led to significant alterations in mycelial morphology, membrane permeability, cellular ultrastructure under the scanning electron microscope, transmission electron microscope and fluorescent microscope, and also retarded the ergosterol amount of fungi. The results confirmed that terpinen-4-ol and 1, 8- cineole act mainly on the organelles and cell membranes of *B. cinerea*, respectively, and when combined possess antifungal activity similar to TTO [24].

4. Antiviral: A previous study suggested that TTO had a promising antiviral activity against Influenza A in MDCK cells. When tested TTO and some of its components, it was found that TTO had an inhibitory effect on influenza virus replication at doses below the cytotoxic dose; terpinen-4-ol, terpinolene, and α-terpineol were the main active components. The results obtained by treating the cells with terpinen-4-ol, terpinolene, and α-terpineol before acridine orange staining exhibited that terpinen-4-ol had a potential role in the anti-influenza virus activity as only in the cells treated with this compound were observed to lack the orange fluorescence. These results were validated by measuring the fluorescence intensity by fluorometry, suggesting that TTO and terpinen-4-ol inhibited acridine orange accumulation in cytoplasmic acid vesicles. Further, the data obtained were consistently by the positive control bafilomycin A1. The acidification of lysosomes in MDCK cells recovered completely when the cells were applied with 0.01% (v/v) TTO for 4h, washed and then incubated for 2 h without the compound showing that the cells can re-acidify after treatment with TTO, and that cell morphology is not affected by treatment. Terpinen-4-ol showed similar results [25].

5. Anti-cancer: The potential anti-tumoral activity of TTO, from *Melaleuca alternifolia,* was tested in an *in-vitro* study against human melanoma M14 WT cells and their drug-resistant counterparts, (M14 adriamicin-resistant cells). Both sensitive and resistant cells were cultured in the presence of TTO (at concentrations ranging from 0.005 to 0.03%). Both complex oil (TTO) and its main active component terpinen-4-ol were able to cause caspase-dependent apoptosis of melanoma cells, and this effect was more profound in the resistant variant cell population. Scanning electron microscopy and freeze-fracturing results suggested that the effect of the oil and terpinen-4-ol was due to their interaction with the plasma membrane and subsequent reorganization of membrane lipids. It was concluded that terpinen-4-ol and TTO can inhibit the growth of human M14 melanoma cells. These appear to be more effective on their resistant variants, which show high levels of P-glycoprotein in the plasma membrane, overcoming resistance to caspase-dependent apoptosis exerted by P-glycoprotein-positive tumor cells [26].

Terpinen-4-ol remarkably improves the effect of several biological and chemotherapeutic agents. It significantly inhibits the growth of pancreatic, colorectal, gastric and prostate cancer cells in a dose-dependent manner (10-90% in 0.005-0.1%). The possible mechanism for its activity involves induction of cell-death rendering this compound as a promising anti-cancer drug alone and in combination in the treatment of numerous malignancies. Terpinen-4-ol restores the activity of cetuximab in cancers with mutated KRAS. Sub-toxic concentrations of terpinen-4-ol synergise anti-CD24 mAb (150 μg/ml)-induced growth inhibition (90%). Considerable retardation in tumor volume was observed following terpinen-4-ol (0.2%) treatment alone and with cetuximab (10 mg/kg) (40% and 63%, respectively) in comparison to the control group [27].

6. Antibiofilm Study: Li-ming Sun, 2012, carried a biofilm evaluation activity for *C. albicans (C. albicans)* ATCC 11231. The antibiofilm activity of terpinen-4-ol-loaded nanoparticles was evaluated against *C. albicans* ATCC 11231 cells by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) method. These nanoparticles resulted in better efficacy toward *C. albicans* ATCC 11231 with a longer duration than did terpinen-4-ol only. The antimicrobial property of the samples against the tested strain was checked with the MIC, MBC, and MBEC. The results exhibited that the mean MIC was 5µg/ml; MBC and MBEC were 10µg/ml. The cellular morphological changes can be observed clearly from SEM images where normal *C. albicans* ATCC 11231 cells grown in YPD medium showed a typical elliptical shape having a smooth surface. The results showed that 10µg/ml terpinen-4-ol effectively eradicated the biofilm. The retardation of *C. albicans* ATCC 11231 metabolic activities was observed after treatment with terpinen-4-ol-loaded lipid nanoparticles. The metabolic activity was 21.5% of control activity, for biofilm exposed to lipid nanoparticles (containing 10µg/ml terpinen-4-ol), and only 9.7% of control activity for terpinen-4-ol exposure (20 µg/ml). Increasing terpinen-4-ol concentration up to 40µg/ml or 80µg/ml showed no additional effect on the kinetics of biofilm eradication [28].

Analysis of Terpinen-4-ol: Analysis of terpinen-4-ol from different plant species is carried out using different analytical methods. In a study, the leaf volatile constituents of the essential oils of *Artemisia indica* Wild and *Artemisia vestita* Wall were studied using a combination of capillary Gas chromatography with flame ionisation detector (GC–FID), gas chromatography-mass spectrometry (GC–MS) and FT-IR (Fourier Transform Infra-Red) spectroscopy. In *Artemisia indica* leaves, terpinen-4-ol (0.4%) was detected using mass spectroscopy and retention index. The percentage was obtained by FID peak area normalization without using response factors [29]. Terpinen-4-ol is the main bioactive in Phlai oil, extracted by steam distillation from the rhizome of *Zingiber cassumunar* Roxb. commonly used as topical anti-inflammatory herbal medicine. An analytical method for assessment, terpinen-4-ol in cutaneous microdialysis samples by GC-MS was developed by Charlock *et al.* The obtained calibration curve demonstrated a linear relationship between the peak area ratio of terpinen-4-ol and methyl salicylate (MeS), which was an internal standard, over a range of terpinen-4-ol 0.36-1.79 ppm. The intra- and inter d precisions at all concentrations tested were less than 1.5% and 4.0% R.S.D, respectively. The recoveries of terpinen-4-ol were in the range of 101.22-111.44%. The analyte was stable in working standard solutions after 40 h at room temperature and in standard stock solutions after three ds at -20 ºC without a relevant loss of signal. The limit of detection and quantification were 0.0294 ppm and 0.0883 ppm, respectively.

The analytical method fulfilled the requirement of method validation followed by International Conference on Harmonisation guideline. However, based on this preliminary evaluation, further method testing of this approach in cutaneous microdialysis should be done. Then, it can be applied to the determination of terpinen-4-ol in topical formulations by using microdialysis model for the dermatopharmacokinetic evaluation [30].

GC-FID or GC-MS was used for analysis of TTO by Mondello *et al.* Gas chromatography equipment used included a Perkin Elmer Auto System having two fused-silica SPB columns (60 m × 0.25 mm i.d.; film thickness 0.25 µm), mounted in parallel in the same oven, with two detectors: FID and Q-Mass 910 (electron ionization 70 eV electron energy, transfer line 220 °C). Carrier gases were oxygen and moisture-free helium obtained from a SUPELCO High Capacity Heated Carrier Gas Purifier (Sigma-Aldrich, Milan), provided with an OMI-2 indicating tube, at an average flow rate of 1 ml/min. The oven temperature programme was kept 60 °C for 4 min, then 2°C/min until 180 °C was reached, then increased 3 °C/min until 250 °C. The detector and the injector temperature was 280 °C. The volume of injected TTO or pure substance was 0.1 µl, and the split ratio was 1:50. Two distinct data systems were connected to the GC-FID or GC-MS: Turbochrom and Q-mass Analytical Workstation Software (PerkinElmer, Milan) with a NIST/EPA/MSDC Mass Spectral database [31].

TTO composition was analyzed by this group by comparing the Kovat's Indices, GC retention times [32], and GC/MS spectra with those of the co-injected reference substances. In the absence of reference substances, the structure of the components was tentatively assigned by the Official NIST/EPA/ MSDL Spectral Library. Quantitative data were based on peak area normalization without using a correction factor. The oil was found to be terpinen-4-ol type according to the European Pharmacopoeia [33] and the International Standard ISO 4730:1996 [34].

GC/MS, chiral GC/MS, and chemometric techniques were used to evaluate a large set (n = 104) of tea tree oils (TTOs) and commercial products containing TTO. Twenty terpenoids were analyzed in each sample and compared with the standards specified by ISO-4730 (2004). Several ISO compliant oil samples when distilled did not meet the ISO standards in this study. This may be primarily because of the presence of excessive *p*-cymene and depletion of terpinenes. Forty-nine percent of the commercial products did not meet the ISO specifications. Four terpenes, *viz.*, α-pinene, limonene, terpinen-4-ol, and α-terpineol, present in TTOs with the (+)-isomer predominant were measured by chiral GC/MS. The results clearly showed that 28 commercial products contained excessive (+)-isomer or contained the (+)-isomer in concentrations below the norms. Of the 28 outliers, 7 met the ISO standards. There was a substantial subset of commercial products that met ISO standards but displayed unusual enantiomeric ± ratios. Based on the oils that met ISO standards, a class predictive model was constructed. The outliers identified by the class predictive model coincided with the samples that displayed an abnormal chiral ratio. Thus, chiral and chemometric analyses could be used to assure the identification of abnormal commercial products including those that met all of the ISO standards[35].

Formulations for Terpinen-4-ol: A limited number of formulations have been reported with terpinen-4-ol in literature. Despite several biological applications, use terpinen-4-ol is limited due to volatile nature, low stability and water-solubility. A solid terpinen-4-ol/β-CD inclusion complex was fabricated using lyophilization by Yang *et al.* Resulting in inclusion complexes of this molecule were found to possess higher thermal stability and sustained release of terpinen-4-ol at high temperatures. To analyse the release kinetics of terpinen-4-ol from its β-CD inclusion complex, avrami's equation was used. The results depicted that the release observed was diffusion based (at 40 °C), and a combination of diffusion and the first-order mechanism was found for other temperatures. Release experiments were carried at various temperatures (40, 60, 80, and 100 °C) with 70 % humidity. The release rate of terpinen-4-ol was remarkably enhanced with an increase in temperature.

Also, better antibacterial activity against *S. aureus, Pseudomonas aeruginosa (P. aeruginosa) and E. coli,* (with concentration ranging from 1.25 mg/ml to 5 mg/ml) was obtained in its inclusion complex. Characterization and evaluation was done using FTIR, XRD, UV-Vis, 1H-NMR, 2D ROESY and TG. In a vacuum, the complexation process of terpinen-4-ol with β-CD was measured by PM3 and ONIOM (B3LYP/6-31G). A 1:1 complex was found to possess an inclusion constant of 252.6.

The TGA results exhibited a significant enhancement in thermal stability of terpinen-4-ol due to the inclusion complexation. The 1H-NMR and 2D ROESY studies ascertained the inclusion and provided information regarding the geometry of terpinen-4-ol inside the β-CD cavity. The thermal stability sustained release and antibacterial activity were enhanced on the inclusion of terpinen-4-ol into β-CD, as affirmed from the quantum chemical calculations and NMR reports. Briefly, terpinen-4-o/β-CD inclusion complex can be promising in the cosmetics, food and pharmaceutical applications. Further, the weak interactions of hydrogen bonds between terpinen-4-ol and β-CD play a significant role in its properties [36].

Terpinen-4-ol shows broad-spectrum antimicrobial activity. However, highly volatile and nonwettability properties have limited its application. Sun *et al.*, 2012, fabricated novel nanocarriers to deliver and protect terpinen-4-ol. The polyethylene glycol (PEG)-stabilized lipid nanoparticles were synthesized and characterized by scanning electron microscope, differential scanning calorimetry, and zetasizer. These nanoparticles after glycine modification had an

average diameter of 397 nm and a zeta-potential of 10. The prepared nanoparticles were having a homogeneous particle size, high drug loading, and stability. Liquid chromatography/mass spectrometry showed a sustained release pattern from terpinen-4-ol nanoparticles. Minimum inhibitory concentration and minimum biofilm eradication concentration were checked against *C. albicans* ATCC 11231. Experiments on isolated mitochondria showed inhibition of enzyme activity and the blockage of biofilm respiration. The effects can be ascertained to the localization of terpinen-4-ol on the mitochondrial membrane [28].

Absorption, Distribution, Metabolism and Excretion Properties: The terpenes cause a modification in the arrangement of lipid in the intercellular region of the stratum corneum (SC) leading to the enhanced skin permeability. This property is commonly used in transdermal drug forms which depend on physicochemical behaviours of terpenes and their concentrations penetrated to the stratum corneum. In a study, four cyclic terpenes, namely terpinen-4-ol, eucalyptol, beta-pinene, and alpha-pinene applied as a neat substance to correlate skin absorption and elimination kinetics with their physicochemical properties. The terpenes were applied *in vitro* onto the human skin, and after 1-4 h their content in the stratum corneum layers and epidermis/dermis separated by tape-stripping method was determined using GC. Similarly, the amounts of terpenes in the skin were also analyzed during 4 h following 1 h absorption. Terpinen-4-ol showed the fastest and progressive penetration into all skin layers.

Although all studied terpenes are absorbed in the viable epidermis/dermis, penetration into these layers was observed as a time-dependent process, which constantly increased during 4 h. For stratum corneum, the largest accumulation for terpinen-4-ol in epidermis/dermis was observed. Further, it was seen that the elimination of terpenes from the stratum corneum was speedy, especially in the deeper layers, and it was much faster if the initial accumulation was small. Investigated cyclic terpenes have depleted different penetration and elimination characteristics. These were not found to permeate across the skin to the receptor medium due to large accumulation in the skin. Results indicated that the penetration of terpenes into stratum corneum is greater if their log P value is close to 3 [37].

In the following year, Cal *et al.,* evaluated the *in-vitro* cutaneous penetration of five terpenes--linalool, linalyl acetate, terpinen-4-ol, citronellol, and alpha-pinene--applied in pure essential oils or in dermatological formulations (o/w emulsion, oily solution or hydrogel) containing the essential oils 0.75% w/w. Variable skin absorption was observed depending on the type of the vehicle and log P values of terpenes. Cutaneous accumulation of terpenes was found several times higher if they were applied as pure essential oils than as topical vehicles. Penetration of terpinen-4-ol through the skin was better from an oily solution (approximately 90 μg/cm (2)) than from an emulsion (60 μg/cm (2)). No penetration of linalyl acetate from topical vehicles into viable skin was seen, but the penetration of this terpene to the upper layers of the stratum corneum was twice higher when an oily solution was used. In contrast, the cutaneous absorption of linalool was observed the same from both vehicles (50-60 μg/cm (2)).

The skin penetration of alpha-pinene was not detectable when it was applied in an oily solution. Only a small amount (approximately 5 μg/cm (2)) of this terpene was measured in viable skin after application as a hydrogel. Citronellol applied as hydrogel penetrated into all skin layers (25 μg/cm) (2), while penetration was absent in viable skin layers after application of an oily solution. The only citronellol permeated into the receptor medium [38].

Another study aimed to explore the effect of excipients conventionally used for topical dosage forms, namely isopropyl myristate or oleic acid or polyethylene glycol 400 or Transcutol, on the human skin permeability of terpinen-4-ol (in the pure TTO) was reported. The effect of such excipients was estimated by evaluating the terpinen-4-ol absorption in human epidermis and the changes of the organization of stratum corneum by ATR-FTIR. Among the tested excipients, oleic acid enhanced the absorption of terpinen-4-ol by modifying the stratum corneum lipid barrier. Other excipients showed a weak permeation enhancement [39].

Chooluck *et al.,* investigated dermal pharmacokinetics of terpinen-4-ol in rats following topical administration of plai oil derived from the rhizomes of *Zingiber cassumunar* Roxb. Unbound terpinen-4-ol amount in dermal tissue was checked by microdialysis. The dermal pharmacokinetic study of terpinen-4-ol was done under non-occlusive conditions. The oil was topically applied at a dose of 2, 4, and 8 mg/cm^2 plai oil corresponding to the amount of 1.0, 1.9, and 3.8 mg/cm^2 terpinen-4-ol, respectively. Following topical application of the oil, terpinen-4-ol gets rapidly distributed into the dermis, and showed linear pharmacokinetics with no changes in the area under the concentration-time curves dose-normalized. The mean percentages of free terpinen-4-ol distributed in the dermis per amount of administered were 0.39 ± 0.06%, 0.41 ± 0.08%, and 0.30 ± 0.03% for 2, 4, and 8 mg/cm^2 doses, respectively. The dermal pharmacokinetics of terpinen-4-ol could prove valuable for its formulation development [40].

To study metabolism, (R)-Terpinen-4-ol was mixed in an artificial diet at a concentration of 1 mg/g, and the diet was fed to the last instar larvae of common cutworm (*Spodoptera litura*). Metabolites were recovered and analyzed spectroscopically. (R)-Terpinen-4-ol was transformed mainly to (R)-*p*-menth-1-en-4,7-diol. In a similar way, (S)-terpinen-4-ol was transformed mainly to (S)-*p*-menth-1-en-4,7-diol. It was observed, that the C-7 position (allylic methyl group) of (R)- and (S)-terpinen-4-ol was preferentially oxidized [41].

Haigou *et al.,* examined the *in-vitro* metabolism of (+)-terpinen-4-ol using human liver microsomes and recombinant enzymes. The biotransformation of (+)-terpinen-4-ol was checked by GC-MS. (+)-Terpinen-4-ol was found to be oxidized to (+)-(1R, 2S, 4S)-1, 2-epoxy-*p*-menthan-4-ol, (+)-(1S, 2R, 4S)-1, 2-epoxy-*p*-menthan-4-ol, and (4S)-*p*-menth-1-en-4,8-diol by human liver microsomal P450 enzymes. The identities of (+)-terpinen-4-ol metabolites were measured through the relative abundance of mass fragments and retention times on GC-MS. Out of 11 recombinant human P450 enzymes checked, CYP1A2, CYP2A6, and CYP3A4 were found to catalyze the oxidation of (+)-terpinen-4-ol. Based on several lines of evidence, CYP2A6 and CYP3A4 were observed to be major enzymes responsible for the oxidation of (+)-terpinen-4-ol by human liver microsomes. Firstly, among 11 recombinant human P450 enzymes tested, CYP1A2, CYP3A4 and CYP2A6 catalyzed (+)-terpinen-4-ol oxidation. Secondly, oxidation of (+)-terpinen-4-ol was inhibited by (+)-menthofuran and ketoconazole, specific inhibitors for these enzymes. Finally, a good correlation was observed between CYP2A6 and CYP3A4 activities and (+)-terpinen-4-ol oxidation activities in the 10 human liver microsomes [42].

Toxicity: TTO was extracted from the Australian native plant, *Melaleuca alternifolia* and its potential anti-inflammatory properties were investigated. The ability of TTO to retard the production *in-vitro* of interleukin (IL)-1beta, tumor necrosis factor-alpha (TNF alpha), IL-8, prostaglandin E2 (PGE2) and IL-10 by lipopolysaccharide (LPS)-activated human peripheral blood monocytes was checked. TTO emulsified by sonication in a glass tube into the culture medium (10% fetal calf serum (FCS)) was toxic for monocytes (at a concentration of 0.016% v/v).

However, the TTO water-soluble components (at concentrations equivalent to 0.125%) remarkably suppressed LPS-induced production of IL-1beta, TNF alpha and IL-10 (by approximately 50%) and PGE2 (by approximately 30 %) after 40 h. Gas chromatography/mass spectrometry confirmed α-terpineol (3%), terpinen-4-ol (42%) and 1,8-cineole (2%) as the water soluble components of TTO. From an individual examination of these compounds, only terpinen-4-ol suppressed the production after 40 h of TNF alpha, IL-8, IL-1beta, PGE2 and IL-10 by LPS-activated monocytes. Hence, the water-soluble components of TTO can suppress pro-inflammatory mediator production by activated human monocytes [43].

The antimicrobial activity of *Melaleuca alternifolia* (tea tree) oil was compared with some of its components, both individually and in two-component combinations. Time-kill assays and MIC revealed that terpinen-4-ol, the major active component of TTO, was more active on its own than when present in TTO. Combinations of terpinen-4-ol and either gamma-terpinene or *p*-cymene showed similar activities to TTO.

Concentration-dependent retardation in terpinen-4-ol activity and solubility was observed in the presence of gamma-terpinene. Non-oxygenated terpenes present in TTO probably lead to the reduction in terpinen-4-ol efficacy by reducing its aqueous solubility. These findings exhibit why TTO can be less active *in-vitro* than terpinen-4-ol alone and further suggested that the presence of a non-aqueous phase in TTO formulations may reduce the microbial activity of its active components [44].

The regulatory properties of the essential oil of tea tree were investigated *in-vitro* by human peripheral blood leukocytes activated. The ability of TTO to reduce superoxide production by neutrophils and monocytes stimulated with N-formyl-methionyl-leucyl-phenylalanine (FMLP), lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA) was explored. The water-soluble fraction of TTO had no remarkable effect on agonist-stimulated superoxide production by neutrophils, but significantly and dose-dependently suppressed agonist-stimulated superoxide production by monocytes. This suppression was not caused by cell death. Chemical analysis assured the water-soluble components as terpinen-4-ol, α-terpineol and 1, 8-cineole. When individually examined, terpinen-4-ol significantly suppressed LPS- and FMLP- but not PMA-stimulated superoxide production; α-terpineol significantly suppressed LPS-, FMLP- and PMA- stimulated superoxide production, 1,8-cineole showed no effect. The results indicated that TTO components suppress the production of superoxide by monocytes, but not neutrophils, suggesting its potential for selective regulation of cell types during inflammation [45].

The combined effect of terpinen-4-ol and capric acid against mycelial growth of *C. albicans* and murine oral candidiasis was investigated *in vitro* and *in-vivo*. Mycelial growth of *C. albicans* was measured by the crystal violet method. Combination of these compounds should be a potent synergistic growth inhibition. Therapeutic efficacy of the combination was also evaluated microbiologically in murine oral candidiasis and results clearly exhibited therapeutic activity. Based on this study, the combination of terpinen-4-ol and capric acid can be used for oral candidiasis therapy [46].

Irritation Study: In addition to increasing reports on therapeutic properties of TTO, several toxicity reviews of TTO have been published. TTO produces local adverse reactions, like contact allergy, irritation, and dermatitis in humans. However, it had been suggested in the literature that level of allergy and skin irritation can be controlled by diluting TTO [47-49].

A study carried in this concern, several diluted concentrations were explored for acute dermal toxicity of TTO. The results demonstrated that skin irritation was remarkably retarded when TTO concentration was less than 2.5%. The TTO components that caused the skin irritation were then further explored. Terpinen-4-ol, the major component in TTO, and 1, 8-cineole, a limited component in TTO, was checked for skin irritation. Results indicated that TTO caused significant skin irritation at 5%, while the maximum percentage of terpinen-4-ol in TTO was 30%. With different concentrations of terpinen-4-ol, no evidence of erythema, edema, or any other skin reactions were seen at 24 h and 48 h.

Hence, the skin irritation of terpinen-4-ol was checked at a concentration of 1.5% and skin irritation was not there. Further, no document describes possible skin toxicity caused by terpinen-4-ol [50].

CONCLUSION: The current trend in research is to promote alternative therapies in addition to conventional therapy as many patients believe these show fewer detrimental side effects. Terpinen-4-ol has a good potential to be developed as an alternative therapy for its promising activities. A wealth of *in-vitro* data supported its antimicrobial, antiviral, anti-biofilm and anti-inflammatory actions. Novel formulations can be fabricated and investigated in future using this molecule to overcome problems like volatility, solubility, and stability. Future, chemists can explore the synthesis of terpinen-4-ol analogs which may provide more effective and therapeutically effective agents in coming future. Still, there is a great need for phytochemical investigations on this bioactive constituent for development of an effective natural remedy responsible for the wide range of applications.

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