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Tea Tree Oil and Its Use in Aromatherapy

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Abstract

Tea tree oil or TTO as it is widely known, is an essential oil with a pale yellow color and distinctive odor, obtained by steam distillation from the leaves of *Melaleuca alternifolia*, which is a native Australian plant. *In vitro* and *in vivo* research have shown that it has mainly, antibacterial, antiviral, antifungal as well as anti-inflammatory properties. TTO is composed of terpene hydrocarbons, namely the main monoterpenes, sesquiterpenes and their associated alcohols. Three of its well-known compounds are 1.8-cineol, α-terpineol and terpinen-4-ol, the latter being the most active one. The ability of these components to disrupt the permeability of cytoplasmic membrane structures of bacteria makes TTO effective on methicillin-resistant bacteria and predominant especially in hospital-acquired *Staphylococcus aureus* infections. In this review historical, and morphological information about *M. alternifolia* plant, chemical properties related to TTO and the mechanism of antimicrobial, antiviral and antifungal actions will be explained, relying extensively on existing literature. Diverse mixtures made of fixed and essential oils relating to TTO that are used as a broad-spectrum antibacterial for some skin infections and acne; for some vaginal fungus infections; for throat infections; for aphthae and cold sore on the basis of the oil's antiviral effect; for skin and nail fungus on the basis of its antifungal property as well as TTO's use in aromatherapy and phytocosmetics will also be illustrated.

Key Words: Aromatherapy, *Melaleuca alternifolia*, TTO, Tea tree, phytotherapy.

1. Introduction

Conventional and complementary therapies have been increasingly used together. Aromatherapy is a relevant complementary therapy and a natural way of utilizing essential oils as therapeutic agents on mood, behavior and ''health''. (Herz R. S., 2009). Their mixtures have been used in traditional healing for centuries. The essential oils spread into the atmosphere by various parts of the plants or evaporation and protect the plant in its natural environment from bacterial attacks or external factors with the aura they form. (Rehman R. et al., 2016; Ali B. et al., 2015).

To have a pharmacological effect, an essential compound has to enter the blood circulation system through the nasal passage, the lung mucosa or the skin.

The signals transmitted to the limbic system via the olfactory nerves make the brain release hormones such as serotonin, endorphin and thereby ensuring control over the central nervous system. The most distinct scientifically proven properties of essential oils are their antibacterial, antiviral, antifungal and anti-inflammatory effects. (Ali B. et al., 2015; Mertas A. et al., 2015).

Essential oils have a wider range of use. For example: it has been shown that the process of administering drugs in accordance with individuals circadian rythms can be

regulated through the use of some essential oils. Research involving the latter's use in diseases such as cancer and Alzheimer are ongoing. (Shibamoto K. et al., 2010).

Essential oils have also applications in cosmetics and dermocosmetics. They are used for skin cleaning, moisturizing, vitalization, rejuvenation, against aging, for eliminating skin blemishes and hair care with formulations prepared by mixing the essential oils in fixed oils in different proportions. (Shibamoto K. et al., 2010; Çakır N. T. et al., 2005).

One of the essential oils with the most expanded indication is TTO. In this study the term TTO will only be used for *M. Alternifolia* oil.

2. History

The Bundjalung people living in eastern Australia knew the traditional use of TTO long before Captain Cook set foot on the Australian shores. They used the TTO, which is extracted by crushing the tree's leaves for coughs and colds through inhalation; the leaf infusion for sore throat and skin diseases; moreover, the crushed leaves that they mixed with clay as massage and rubbing material. They utilized the lagoons formed by the rotten leaf sediments as healing pools. As there were no scientific data proving the effectiveness of *M. alternifolia*, the Westerners considered it as a "bush medicine" for a long period. The white people who settled first in that region have examined and learned how to use the leaves for their own healing applications. (Carson C.F. et al., 2006; Carson C.F. et al., 1993; Çakır N. T. et al., 2005).

The use of TTO in industry has begun after the announcement by Penfold that the antimicrobial effectiveness of *M. alternifolia was* shown to be eleven-fold compound Phenol's. Upon the extensive studies they realized after obtaining TTO through distillation, Arthur Penfold and his team have affirmed antibacterial and antifungal effects of the oil and that it is a new antimicrobial, which is effective against microbes and further moregentle on skin cells. (Carson C.F. et al., 2006; Mertas A. et al., 2015).

In World War II the Australian soldiers had this oil in their first aid kits. (Sharifi J.R. et al., 2017). In fact, bush cutting laborers were exempted from national service due to the intensity of demand for this oil. (Sharifi J.R. et al., 2017). The fame of the essential oil of *M. alternifolia* grew day by day and TTO became the most preferred healing product in all kinds of bacterial infections as well as many other cases including cold, canker sores, cold sores, tonsillitis, head lice, alopecia, leg ulcers, pyorrhea and gingivitis. (Ali B. et al., 2015). At present *M. alternifolia* which is endemic to Australia and also introduced and cultivated in other continents is one of the most known species. (Sharifi J.R. et al., 2017).

After the war the use of natural products as antimicrobials has decreased considerably following the discovery of antibiotics, the oil's production has been negatively affected by this and it has been forgotten during approximately 20 years. The emergence of the interest for essential oils and natural products in the plantations led to large-scale essential oil production (Carson C.F. et al., 2006). The examination of the principal components of the antimicrobial activity of the *M. alternifolia* oil by scientists at the beginning of the 1990s has demonstrated that this oil is resistant to Meticillin and noticeably sensitive to *Staphylococcus aureus* or MRSA, the ''superbug'' found in hospitals and whose effect has been increasing. In fact the MRSA incidence in US and European hospitals had risen from 3 % in the 1980s to 40 percent at the end of the 1990s. The superbug had become the major problem for persons with skin lesions and especially those with postoperative wounds whose immune system was suppressed. The knowledge obtained from studies realized in the latter years showed that TTO can be

used as a natural antimicrobial agent against *S. aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Aeromonas hydrophila, Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis and Klebsiella pneumonia.* (Mumu S. K. et al., 2018).

3. Botanical Features and Nomenclature

M. alternifolia belongs to Equisetopsida class, Magnoliiadae subclass, Rosanae superorder, Myrtales order, *Myrtaceae* family, and Melaleuca genus of the plant kingdom (Sabir S. et al., 2014). It grows on the mild, humid coasts of Eastern Australia at 300 m altitude where the maximum summer temperature is 27-31 0C, the minimum winter temperature 19-21 0C the average precipitation 1000-1600 mm on soils with a pH of 4.5-7. It is a tall shrub reaching 7m, growing in dense swamps and with a layered and papery bark. *M. alternifolia* is densely flowered and generally blooming in October and November. Its flowers are produced in loose, white to creamy colored terminal spikes, which can give trees a "fluffy" appearance. (Carson C. F. et al., 2006).

In each bud there is a calix tube of up to 3mm, petals 3mm long, 30-60 stamen, a pistil with a 3mm long stilus and a stigma. Its fruit is many seeded, cup shaped and 2-3mm in diameter. It has a hole of 1.5-2.5mm diameter which enables the release and dispersal of seeds by the wind. (Carson C.F. et al., 2006; Sabir S. et al., 2014). Tea Tree Oil is an oil known by many very different names including ''TTO'', ''Melaleuca Oil'' or ''Indian Daphne'.' As ''Tea Tree Oil'' is the common name used in the Maori and Samoa languages for plants of the Cordyline genus and as oils with chemical compositions differing from each other are obtained from other Melaleuca species as well, the term ''Tea Tree Oil'' remains insufficient for expressing Tea Tree Oil. (Sabir S. et al., 2014; Carson C. F. et al., 2001). In fact, those oils outside *M. alternifolia* shown in Table 1. are used as well for obtaining TTO. In addition to these, the essential oils Kanuka and Manuka distilled from the New Zealand plants *Kunzea ericoides* and *Leptospermum scoparium* are also known as TTOs and do not differ much from the Australian TTO. (Carson C.F. et al., 2006; Carson C. F. et al., 2001).

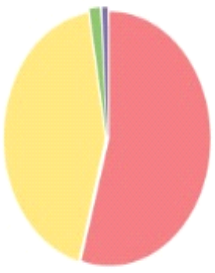
Table 1. Melaleuca species and their common names

M.alternifolia	Tea tree, TTO,
M. linariifolia Smith	TTO, tea tree
M. dissitiflora F. Muell.	TTO, tea tree
M. cajuputi Powell	Kajeput, swamp tea tree, paperbark tree
M. quinquenervia	Niaouli, broad-leaved tea tree, broad-leaved paperback tree
M. viridiflora	TTO, Tea tree
Leptospermum scoparium	Manuka
Kunzea ericoides	Kanuka

In Australia, another name used for "tea trees" is "paperbark trees". All of these terms can refer to up to several hundred species belonging to the Melaleuca or Leptospermum genus. For example, the common names for *M. cajuputi* are "swamp tea tree" and "paper bark tea tree" while those for *M. quinquenervia* include "broad-leaved tea tree" and "broad-leaved paper bark" (Sabir S. et al., 2014). Several ornamental species of Leptospermum are often mistakenly identified as the source of TTO. Therefore the common plant names used can render the issue complicated. **(Table -2)** According to statistics the Melaleuca genus comprises 451 scientific species names and 246 of these are accepted species names (The plant List 2018).

Table 2. Given names for Maleleuca genus

Status	Total	Percentage %
Accepted	246	54.5
Synonym	195	43.2
Unplaced	7	1.6
Unassessed	3	0.7



4. Essential Oil Chemotypes and Their Chemical Composition

The quantity of the individual terpenes in TTO can vary considerably depending on the Melaleuca population and chemotype used, climate, age of leaves, leaf maceration and time of distillation. The most abundant of terpenes is terpinen-4-ol, which has a significant role in the oil's antimicrobial activity (Sharifi J. R. et al., 2017). Some researchers have reported that an optimal balance between the terpinen-4-ol and 1.8 cineol was needed as this was necessary for both the antimicrobial and skin penetration particularities of TTO. It has been indicated that the terpinen -4-ol content needs to be above 30 % in order to optimize antimicrobial activity. On the other hand, as it is known that skin and mucosa irritation effects of TTO due to long term or high dosage usage stems from 1.8-cineole content, which should not exceed 15 % (Mertas A. et al., 2015). Oil yields and compositions for various Melaleuca species are given in Table 3.

Table 3. Oil yield and content in various Melaleuca species

	M. alternifolia	*M. cajeputi*	*M. quinquenervia*	*M. bracteata*	*M. linariifolia*	*M. dissitiflora*
Oil yield in fresh plant	1-2 %	0,4-1,2 %	0,5-2,5 %	Below 1 %	1,5-4,0 %	1,4-4,2 %
Terpinen-4-ol	40,1 %	-	-	-	32-42 %	30-52 %
α-terpinen	10,4 %	-	-	-	8-12 %	6-10 %
γ-terpinen	23 %	-	-	-	18-25 %	13-20 %
1,8-cineol	5,1 %	3-60 %	35-76 %	-	4-8 %	3-15 %
Viridiflorol	0,1 %	0-16 %	17-76 %	-	-	-
Globulol	0,2 %	0-9 %	-	-	-	-
Spathulenol	-	0-30 %	-	-	-	-
Nerolidol	-	-	43-95 %	-	-	-
Methyl eugenol	-	-	-	Up to 90 %	-	-
Phellandrene	-	-	-	Up to 5 %	-	-
Terpinolen	3,1 %	-	-	-	-	-
P-cymene	2,9 %	-	-	-	-	-
α-pinene	2,6 %	-	-	-	-	-
α-terpineol	2,4 %	-	-	-	-	-
Aromadendrene	1,5 %	-	-	-	-	-
Cadinene	1,3 %	-	-	-	-	-
Limonene	1 %	-	-	-	-	-
Sabinene	0,2 %	-	-	-	-	-

4.1. Composition and Chemistry

TTO whose chemical composition is well defined is composed of terpene hydrocarbons, namely the main monoterpenes, sesquiterpenes and their associated alcohols. Most of the monoterpenes are cyclic, 50 % are oxygenated and 50 % are composed of hydrocarbons. (Carson C. F. et al., 2006). TTO has a density of 885-906 kg/m3, a boiling point of 176 **0**C, a refractive index of 1.475-1.482, an optical rotation of 5 to 15 degrees, is sparingly soluble in water and miscible with nonpolar solvents. (Sabir S. et al., 2014; SCCP 2008).

The 14 components of TTO are regulated by an international standard (for "Oil of *Melaleuca*- terpinen-4-ol type"). The standard does not impose the species of *Melaleuca* from which the TTO are required to be sourced; it indicates physical and chemical criteria for the desired chemotype. (Carson C. F. et al., 2006). Each of the 6 chemotypes described produce oil with a distinct chemical composition. These include a terpinen-4-ol chemotype, a terpinolene chemotype and four 1,8-cineole chemotypes. The main components of TTO are specified in Table 4 by the Standard of the International Organisation for Standardisation (ISO 4730) and European Pharmacopoeia (EP) Monograph (Mertas A. et al., 2015).

Table 4 Comparison of the ratio of main components in TTO based on ISO and the EP Monograph

Components	ISO	European Pharmacopoeia
terpinen-4-ol	30-48	Minimum 30 %
γ-terpinen	10-28	10-28 %
1,8-sineol	Up to 17%	Less than 15 %
α-terpinen	5-13 %	5-13 %
α-terpineol	1,5-8 %	1,5-8 %
p-simen	-	0,5-12 %
α-pinen	-	1-6 %
terpinolen	-	1,5-5 %
aroma-dendren	-	Less than 7 %
limonen	-	0,5-4 %
sabinen	-	Less than 3,5 %

4.2. Antimicrobial Components of TTO

Initial findings obtained from Rideal-walker (RW) coefficients when looking for TTO components responsible for antimicrobial activity showed that most of them are from terpinen-4-ol and α-terpineol. Present data confirms that these two components contribute significantly to the oil's antibacterial and antifungal activities. (Carson C. F. et al., 2006)

4.3. Antibacterial Activity

The lipophilic nature and low molecular weights of essential oil components allow them to penetrate cell membranes, change cell layers, enhance membrane fluidity, furthermore, cause the leakage of ions and cytoplasmic content. (Giacinto B. et al., 2016). When it was shown that these materials damage the vital functions on the biologic membrane, it was accepted that TTO too destroys these functions and has an antimicrobial effect (Sharifi J. R. et al., 2017). Antimicrobial screening, minimum inhibitor concentration and minimum bactericidal concentration analyses have shown that the essential oil obtained from *M. alternifolia* strongly inhibits the growth of various microorganisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Penicillium italicum Wehmer, Penicillium digitatum Sacc. Tus.* (Zhang X. et al., 2018). Moreover, studies have shown that treating *Staphylococcus aureus* with TTO results in the leakage of potassium ions and of 260 nm. light absorbing materials. Therefore, inhibits respiration. (Carson C. F. et al., 2002). The studies demonstrated that no antibiotic resistance developed in the tested *S. aureus, S. epidermidis* and *E. coli* strains. Additionally, that repeated treatment with terpinen-4-ol did not decrease antimicrobial susceptibility. (Hammer K. A. et al., 2012). In the evaluation of the antimicrobial activity of *M. alternifolia* and other oils, comparisons with the disinfectant carbolic acid or phenol were made using the RW coefficient test. The activity of TTO turned out to be 11 fold higher compared with that of phenol. The RW coefficients of several other TTO components measured included 3.5 for cineole, 8 for cymene, 13 for linalool, and 13.5 for terpinen-4-ol and 16 for terpineol. Accordingly, TTO was promoted as a therapeutic agent, which is a denomination that it deserves. (Carson C.F. et al., 2006).

In the examination of antibacterial activity with the Broth Dilution and Agar Well Diffusion methods against the 10 pathogenic bacteria *S. aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Aeromonas hydrophila, Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis, Klebsiella pneumonia* and *Streptococcus agalactiae* in a study it was concluded that TTO is completely effective against all of them. (Sharifi J. R. et al., 2017).

In the conclusion of a study on preventing the potential destructive consequences of an uncontrolled spread of MRSA, TTO has been proposed as an alternative topical decolonization agent. (Brady A. et al., 2006)

Another study measuring the effects of TTO on acne, it has shown that the application of gel products comprising 5% tea tree oil decreases the number of lesions in persons with mild to moderate acne. Comparative studies have shown that TTO products are more effective than the placebo, that they are equivalent to products containing 5 % benzoyl peroxide and 2 percent topical erythromycin and that their side effects appear in similar ratios compared to the products experimented in other topical treatments. It has been estimated that its effectiveness is due to its antibacterial and anti-inflammatory activities (Hammer K.A., 2014; Carson C. F. et al., 1993).

In conclusion, the membrane integrity, its function and the intracellular material of the bacterium were lost and accordingly, insufficient protection of homeostasis led to the death of the bacterium. (Carson C. F. et al., 2006).

4.4. Antifungal Activity

While the susceptibility of fungi to TTO applications was known as being almost limited to *Candida albicans,* new data show that a range of yeasts, dermatophytes, and other filamentous fungi are also susceptible to TTO. (Carson C. F. et al., 2006).

The treatment of *C. albicans* with 0.25 TTO resulted in the absorption of propidium iodide after
30 min and considerable dying with methylene blue and loss of 260-nm-light-absorbing materials after 6 h. Further research shows that treating *C. albicans* with 0.25% TTO increases substantially membrane fluidity and this confirms that the membrane characteristics of *C. albicans.* TTO also inhibits respiration in *C. Albicans* in a dose-depending manner. Treatment with 1.0% and 0.25% TTO inhibited respiration by approximately 95 percent and 40 percent respectively. The respiration of *Fusarium solani* inhibited by 50% with 0.023% TTO. TTO inhibits glucose-induced medium acidification by *C. Albicans*, *C. glabrata*, and *Saccharomyces cerevisiae* as well. This acidification happens mainly by the expulsion of protons by the plasma membrane ATPase, which is fuelled by ATP derived from the mitochondria. The inhibition of this function implies that the plasma and/or mitochondrial membranes have been negatively affected. These results are compatible with a proposed mechanism of antifungal action according to which TTO causes changes or damage to the functioning of fungal membranes. (Carson C. F. et al., 2006).

In a study on 28 women suffering from vaginitis due to *C. albicans,* after treatment with vaginal pessaries with 0.2 g of TTO, 21 were without *C. albicans* and 24 without burning sensation. In another study on 130 women with cervicitis or vaginitis due to *Trichomonas vaginalis* or vaginitis in turn due to *C. albicans*, 40 percent emulsified solution of TTO was effective in their treatment (Sabir S. et al., 2014). The antiviral activity of TTO was first shown by using the tobacco mosaic virus and tobacco plants. In field trials with *Nicotiana glutinosa*, plants were sprayed with 100, 250 or 500 ppm TTO

or control solutions and they were experimentally infected with the tobacco mosaic virus. After 10 days, the plants treated with TTO were found to have considerably less lesions per square centimeter of leaf compared to controls. As a result of these studies, TTO has been observed to be effective against enveloped and non-enveloped viruses, although the range of viruses tested until now has been very limited. (Bishop C. D. 1995).

4.5. Antiviral Activity

The most common viral infections in humans are caused by *Herpes simplex* viruses (HSV) (Type-1 and Type-2), (HSV-1 and HSV-2), which can have fatal consequences. Although synthetically produced medicines are used against Herpes viruses, the desired results cannot be obtained in genital infections. Moreover, the HSV-1 and HSV-2 viruses are also developing resistance to antiviral medicines (Edris A.E., 2007). Scientists have researched the effects of TTO by incubating viruses with various concentrations of TTO and next using these treated viruses to infect cell monolayers. After 4 days, the numbers of plaques formed by the treated virus and the controls were determined and compared. The concentration of TTO inhibiting 50% of plaque formation was 0.0009% for HSV type 1 (HSV-1) and 0.0008% for HSV-2, compared to controls. These studies also indicated that at higher concentration of 0.003%, TTO decreased HSV-1 titers by 98.2% and HSV-2 titers by 93.0% (Carson C. F. et al., 2006).

Consequently, essential oils appear as an alternative method for replacing antiviral medicines. Moreover, their low toxicity risk provides an advantage in connection with the use of plant products.

4.6. Antiprotozoal Activity

Trypanosoma and *Leishmania* are parasitic prozotoa which affect millions of people in the world. At present there are only a small number of medicines with severe side effects for the treatment of Protozoa infections. The literature shows that secondary plant compounds like phenols, chalcones and flavonoids display antiparasitic activity against the *Leishmania, Trypanosoma* and *Plasmodium* species. On the other hand, studies have shown that Terpinen-4- ol which is the main compound of TTO is 1000 times more toxic to trypanosomes than to human cells (Mikus J. A. Q. et al., 2000).

In another study it has been demonstrated that, TTO at 300 mg/ml destroys all cells of *Trichomonas vaginalis* and that it can be effective in the treatment of such infections (Carson C. F. et al., 2006). In some recent studies it has been shown that TTO is effective against Demodex parasites. These are among the most common parasites seen in humans and usually cause ocular surface inflammation. Of the two types of Demodex parasites encountered in the eyes and eyelashes one is often situated in the eyelash follicles and the other in the sebaceous glands. It has been indicated that TTO and especially its main component terpinen-4-ol is effective in reducing mite counts and thereby ocular surface inflammation associated with blepharitis, conjunctivitis, keratitis and chalazion, when used in eyelid cleaning at different concentrations. (Lam N. S. K. et al., 2018).

4.7. Antitumor Activity

It has been advanced that TTO's effect on tumor cells is mediated by its induction of a reorganization of the plasma membrane's lipid architecture and demonstrated that topical 10% TTO/dimethyl sulfoxide (DMSO) substantially delays the growth of subcutaneous melanomas (Pazyar N. et al., 2013).

4.8. Effect of TTO on the Membrane Integrity

The cytoplasmic membranes of bacteria as well as the plasma and mitochondrial membranes of yeast constitute a barrier to the passage of small ions and permit cells and organelles to control the entry and exit of different compounds. This permeability barrier role of cell membranes is integral to many cellular functions; these comprise the maintenance of the cell's energy status, other membrane-coupled energy-transducing processes, solute transport, metabolism regulation and control of turgor pressure. Toxic effects on membrane structure and function have generally been used to explain the antimicrobial action of essential oils and their monoterpenoid components. A large number of studies have shown that monoterpenes damage the cell membrane. The examination of *E. coli* cells with the electron microscope revealed the coagulation of the compounds in the cytoplasm and intensive electron loss in the cell. As these are secondary events appearing after cell death, it has been shown clearly that TTO inhibits *E. coli* cell respiration in suspensions in which it has a lethal effect connected to cytoplasmic membrane damage by stimulating the passage of potassium ions (Cox S.D. et al., 2000). Several studies have shown that TTO obstructs cell growth and causes cell death in the*E. Coli, S. aureus* and *C. albicans* species by changing their membrane permeability. (Carson C.F. et al., 2002)

4.9. Anti-Inflammatory Activity

The anti-inflammatory activity of TTO is confirmed by the evidence provided by a large number of recent studies. It has been shown in the last decade that TTO affects a spectrum of immune responses, *in vitro* as well as *in vivo*. Terpinen-4-ol, α-terpineol, and 1,8-cineole were identified as the main components, but only terpinen-4-ol among them reduced the production of TNF-α. Upon the examination of TTO's effect on hypersensitivity reactions linked to mast cell degranulation in mice it was shown that TTO and terpinen-4-ol applied after histamine injection reduced histamine-induced skin edema. It has also been observed that flare linked with nickel-induced contact hypersensitivity and erythema in humans are also decreased by neat TTO. Other studies have demonstrated that terpinen-4-ol modulates the vasodilation and plasma extravasation linked to histamine-generated inflammation in humans (Carson C.F. et al., 2006).

4.10. Antimicrobial Resistance to TTO

Although the oil has been used in Australia since the 1920s, no clinical resistance against TTO has been reported. In the period up to the 1970s there has been a significant decrease in mortality and morbidity connected to infectious diseases and this period has been considered as the golden era of the discovery of antibiotics. However, in the following decades bacteria able to resist more than one antibiotic started to spread

and this has led to the rise of mortality, morbidity and healthcare costs. Factors such as the misuse of antibiotics in the treatment of diseases and the widespread use of antibiotics in animal husbandry contributed to the emergence of antibiotic resistance. Expanding worldwide travelling and movement of patients have also led to an increase in the transmission of organisms from one country to another. The extensive resistance against antibiotics observed constitutes at present a most serious public health concern and medical experts have started to warn humans about a return to the pre- antibiotic era (Gupta P. D. et al., 2017).

Existing studies have shown that TTO has very little effect toward the development of antibiotic resistance and that treatment with its main component terpinen-4-ol does not change significantly antimicrobial susceptibility (Hammer K. A. et al., 2012). The multicomponent characteristic of TTO might by its nature be making spontaneous resistance formation less likely since multiple simultaneous mutations may be needed to overcome all of the antimicrobial actions of each of the components (Carson C.F. et al., 2006).

4.11. Safety and Toxicity

While studies on the safety and toxicity of the TTO are insufficient, the evidence based on its safe use for almost a hundred years shows that its topical use is safe and its side effects small, limited and rare. Oral and dermal toxicities of TTO were summarized briefly below.

Oral Toxicity

Essential oils are eliminated from the body via the kidneys instead of the liver. While this provides selectivity in the treatment, essential oils, which are unsuitable or not used in the right dosage can lead to toxicity. Cases of involuntary poisoning after an intake of TTO show that at relatively high dosages TTO leads to central nervous system depression and muscle weakness; these symptoms had generally disappeared within 36 h in the cases where they appeared (Sharifi J. R. et al., 2017).

Sufficient knowledge about the safety of TTO use during pregnancy and lactation is lacking. The literature does not mention cases of human death linked to TTO, however, oral use is contraindicated. No indication should suggest oral use until more advanced scientific studies are realized on TTO's toxicity.

Dermal Toxicity

If we leave the olfactory system aside, essential oils enter the blood circulation system via the skin and mucosa in 30 seconds and show their effects in 4-12 minutes. Attaining maximum concentration in 20 minutes, they reach the sick organs and areas through the blood circulation system and relevant nerves and lead to systemic effect (Pazyar N. et al., 2013). The quantity of essential oil absorbed varies according to the part of the body on which it is used, the temperature of the body, the integrity of the skin, the age of the skin, the ambient temperature, and also the proportion of essential oil diluted, the quantity covering the skin's surface, the chemical structure of the oil and the choice of the carrier (lotion, oil, gel, creme, alcohol, water) (Buckle J, PhD, RN., 2016). The lipophilic nature of TTO, which allows it to penetrate the skin's surface layer does not only increase antimicrobial effects but also TTO toxicity linked to dermal absorption (Mertas A. et al., 2015). Although it has side effects such as skin irritation, allergic contact dermatitis, systemic contact dermatitis, linear immunoglobulin-A disease,

erythema multiform reactions, systemic hypersensitivity reactions and idiopathic male prepubertal gynecomastia in parallel with the increase in the use of TTO, it is important that TTO and its components are not mutagenic and genotoxic (Mertas A. et al., 2015; Pazyar N. et al., 2013).

TTO causes irritation and it can also lead to allergic reactions. Patch test results of 311 volunteers showed an average irritancy score of 0.25 for neat TTO. In another study in which a patch test with 10% TTO was applied to 217 patients, no irritant reaction was observed; it was possible to prevent irritant reactions by using lower concentrations of the irritating product. Although it has been claimed that a series of compounds are responsible for some reported allergic reactions, it has also been pointed out that these are due to oxydation products made of obsolete or improperly stored oils (Carson C. F. et al., 2006).

5. Selected Volatile Oil Based Formulas Comprising TTO Used in Aromatherapy

Volatile oils obtained from the flowers, leaves, stems, fruits and roots of plants or distilled from their resins and comprising compounds with a therapeutic value are used in aromatherapy. One of the most important essential oils used in aromatherapy is TTO. It is deservedly included in formulas prepared by pharmacists and used in various diseases. As indicated in the table below the external use of a few drops TTO is effective in the treatment of several diseases thanks to its antimicrobial effect.

The examples given in the following table are for indicative purposes. They need to be applied under the control of a doctor or a pharmacist expert in this domain.

Table 5. Common preperations including TTO

EFFECT	DOSE	USE
Apthae and cold sores	TTO 2-3 drops	Dripped onto a cotton bud which is held on the area for 15 seconds Applies 3-4 times a day. Swallowing not recommended
Acne	TTO 2 -3 drops	Held on the acnes for 15 seconds with the help of a cotton bud, applied 2-3 times a day
Athlete's foot	TTO 2-3 drops	Held 15 seconds on the infected, part, can be applied 3-4 times a day
Foot sweating problem	TTO 10 drops Cypress essentialoil 8 drops	The mixture is mixed with hot water until the feet are covered The foot is left to rest for 15 minutes
Soar throat and hoarseness	TTO 3-4 drops	Mouthwash upon adding it to a tea glass full of drinking water. Is repeated 3-4 times a day. Not to be swallowed
Tooth ache	TTO 3 drops Mint essentialoil 3 drops	Mouthwash upon adding 3 drops of TTO, 3 drops of mint leaf essentialoil into one or half a tea glass.
Halitosis	TTO 5 drops, Medical camomile 1 drop or Mint oil 1 drop	Mouthwash upon adding it to a tea glass of tepid water
Hair eczema and dandruff	TTO 80 drops, Cypress essentialoil 80 drops	Added to 200ml organic shampoo
Vaginal fungus infection	TTO 5 ml, Distileled water 100 ml	5 ml TTO is added to 100 ml distilled water, external washing is applied
Bed sores	TTO2 ml Lavender oil 2 ml, common St-John's wort oil 50 ml	The mixture is applied several times a day to the infected area

6. Conclusion

M. alternifolia oil, or TTO, one of the most valuable essential oils with its antibacterial, antiviral, antifungal, anti-inflammatory and additional particularities. Used as single or in combination with other compounds/oils in complementary treatments, its therapeutic effects which have been confirmed by scientific research confer its reliability. A particularly critical aspect related to the subject is that in the latter period the development of antibiotic resistance has been causing grave concern throughout the world. Several drug companies have slowed down or abandoned antibiotic production. A number of essential oils obtained from plants disrupt the cell membranes of bacteria, viruses and fungi, consequently they cause efflux pump or quorum sensing inhibition. In this framework, the intensity of the effect against antibiotic resistance exhibited by essential oils by themselves or in combination with antibiotics in the studies realized in this field is promising. The anticipated and still unknown therapeutic effects of these and similar developments are expected to benefit greatly from and be revealed by new studies to be realized in this domain.

M. alternifolia oil is one of the most potent and promising essential oils in the context of potential progress in this vein. One of the major aims of this paper is to contribute to the stimulation of further warranted research in this direction.

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