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Review paper

ANTIMICROBIAL, ANTIOXIDANT AND ACARICIDAL PROPERTIES OF TEA TREE (*Melaleuca alternifolia*)

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ABSTRACT: Aim of this paper is to show antimicrobial, antifungal, antioxidant and acaricidal properties of tea tree (*Melaleuca alternifolia*). Tea tree exhibits wide spectrum of antimicrobial activity. Its mode of action against the Gram-negative bacterium *Escherichia coli*, the Gram-positive bacterium *Staphylococcus aureus*, and the yeast *Candida albicans* has been investigated using a range of different methods. As antimicrobial, tea tree possess high inhibitory antifungal activity because of its components such as terpinen-4-ol, α-terpineol, linalool, α-pinene, β-pinene, and β-myrcene followed by 1,8-cineole. Bioactive compounds such as α-terpinene, α-terpinolene, and γ-terpinene shows high antioxidant activity of tea tree. Also, tea tree with its components are known to possess bacteriostatic and germicidal properties and are used to cure infections of the skin and mucous membranes such as boils, abscesses and onychomycosis caused by *Candida*. Their apparent insecticidal and acaricidal properties have to date been tested on some human ectoparasites such as head lice and *Sarcoptes scabiei*, var. *hominis*, and in recent time tea tree extracts were used in research for controlling ticks (*Ixodes ricinus*) that are efficient vectors of pathogens.

Key words: *tea tree, Escherichia coli, Staphylococcus aureus, ROS, ectoparasites, Ixodes ricinus*

INTRODUCTION

Melaleuca alternifolia, commonly known as tea tree (Holliday, 2004) is a species of tree or tall shrub in the myrtle family, Myrtaceae. Endemic to Australia, it occurs in southeast Queensland and the north coast and an adjacent range of New South Wales where it grows along streams and on swampy flats, and is often the dominant species where it occurs. *Melaleuca alternifolia* is a small tree to about 7 m with a bushy crown and whitish, papery bark. The leaves are arranged alternately, sometimes scattered or whorled. The leaves are smooth, soft, linear in shape, 10–35 mm long and 1 mm wide. They are also rich in oil with the glands prominent. Flowers occur in white or cream-colored masses of spikes 3–5 cm long over a short period, mostly spring to early summer, and give the tree an appearance of looking fluffy. The small woody, cup shaped fruit, 2–3 mm in diameter are scattered along the branches (Holliday, 2004). Tea tree has been used as an alternative medicinal treatment for almost a century in Australia and is now becoming more commonplace all around the world. The indigenous people of all around the world use tea trees as a traditional medicine by inhaling the oils from the crushed leaves to treat coughs and colds. They also sprinkle leaves on wounds, after which a poultice is applied. In addition, tea tree leaves are soaked to make an infusion to treat sore throats or skin ailments (Shemesh and Mayo, 1991; Carson et al., 2006). Characteristic of the myrtle family Myrtaceae, it is used to distil essential oil. It is the primary species for commercial production of tea tree oil, a topical antibacterial (Carson et al., 2006). Tea tree oil is commonly used as a topical antiseptic agent because of its antimicrobial properties, especially in the treatment of acne (Hammer et al., 2006) It is also known to reduce inflammation and may be effective in the treatment of fungal infections such as Athlete's foot (Hammer et al., 2006). Tea tree oil should not be ingested in large amounts due to its toxicity and may cause skin irritation if used topically in high concentrations. No deaths have been reported in medical literature was also reported in research of Hammer et al. (2006).

Having in mind aforementioned, the aim of this review paper was to collect and summarized information and to show antimicrobial, antioxidant and acaricidal properties and effects of tea tree (*Melaleuca alternifolia*).

ANTIMICROBIAL PROPERTIES OF TEA TREE

Melaleuca alternifolia, commonly known as tea tree, has a long history of use as a topical antiseptic (Markham, 1999). In recent times it has gained a reputation as a safe, natural and effective antiseptic. This has led to resurgence in popularity and currently it is incorporated as the principal antimicrobial or as a natural preservative in many pharmaceutical and cosmetic products intended for external use. The chemical composition of tea tree oil has been well defined and consists largely of cyclic monoterpenes (Brophy et al., 1989) of which about 50% are oxygenated and about 50% are hydrocarbons. Tea tree oil as a main component (Table 1) exhibits broad spectrum antimicrobial activity which can be principally attributed to terpinen-4-ol (Southwell et al., 1993; Carson and Riley, 1995). A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active monoterpene constituents. Several studies have also shown that monoterpenes exert membrane damaging effects. Examination of *Escherichia coli* cells using electron microscopy after exposure to tea tree oil revealed a loss of cellular electrondense material and coagulation of cytoplasmic constituents, although it was apparent that these effects were secondary events that occurred after cell death (Gustafson et al., 1998). Tea tree oil also stimulates leakage of cellular potassium ions and inhibits respiration in *E. coli* cell suspensions, providing evidence of a lethal action related to cytoplasmic membrane damage (Cox et al., 1998). Cox et al. (2000) in their research have reported the results of the antimicrobial activity of tea tree oil against three clinically significant microorganisms, *E. coli*, *S. aureus* and *C. albicans*. In their study (Cox et al., 2000), tea tree oil inhibited respiration in *E. coli*, *S. aureus* and *C. albicans* cells at minimum inhibitory levels. The possibility that tea tree oil directly inhibits a specific respiratory enzyme or metabolic event cannot be eliminated according the authors, but their findings also reveal that minimum inhibitory levels of tea tree oil altered cell membrane structure. Increased uptake of the nucleic acid stain propidium iodide, to which the cell membrane is normally impermeable, was observed. Also, leakage of potassium ions commenced immediately upon adding tea tree oil to suspensions containing *E. coli* and within 5 min for *S. aureus* cells.

Table 1. Tea tree oil composition, %

Content	Concentration
terpinen-4-ol	35.0–48.0
γ-terpinene	14.0–28.0
α-terpinene	6.0–12.0
1,8-cineole	traces–10.0
terpinolene	1.5–5.0
α-terpineol	2.0–5.0
α-pinene	1.0–4.0
p-Cymene	0.5–8.0
Sabinene	traces–3.5
limonene	0.5–1.5
aromadendrene	0.2–3.0
ledene	0.1–3.0
globulol	traces–1.0
viridiflorol	traces–1.0

Study of Hammer et al. (2011) examined the effect of subinhibitory *Melaleuca alternifolia* (tea tree) essential oil on the development of antibiotic resistance in *S. aureus* and *E. coli*. Frequencies of single-step antibiotic-resistant mutants were determined by inoculating bacteria cultured with or without subinhibitory tea tree oil onto agar containing 2 to 8 times the MIC of each antibiotic and with or without tea tree oil. Whereas most differences in resistance frequencies were relatively minor, the combination of kanamycin and tea tree oil yielded approximately 10-fold fewer resistant *E. coli* mutants than kanamycin alone. The development of multistep antibiotic resistance in the presence of tea tree oil or terpinen-4-ol was examined by culturing *S. aureus* and *E. coli* isolates daily with antibiotic alone, antibiotic with tea tree oil and antibiotic with terpinen-4-ol for 6 days. Median MICs for each antibiotic alone increased 4- to 16-fold by day 6. Subinhibitory tea tree oil or terpinen-4-ol did not greatly alter results, with day 6 median MICs being either the same as or one concentration different from those for antibiotic alone. For tea tree oil and terpinen-4-ol alone, day 6 median MICs had increased 4-fold for *S. aureus* and 2-fold for *E. coli* from baseline values. Lastly, Hammer at al. (2011) seen few significant changes in antimicrobial susceptibility for *S. aureus* and *S. epidermidis* isolates that had been serially subcultured 14 to 22 times with subinhibitory terpinen-4-ol, so these data indicate that tea tree oil and terpinen-4-ol have little impact on the development of antimicrobial resistance and susceptibility. *Paenibacillus* larvae and *Melissococcus plutonius* are the primary bacterial pathogens of honeybees and the causative agents of American and European foulbrood disease respectively. Such diseases have been gaining importance since there are few therapeutic options beyond the reporting of microorganisms resistant to conventional antibiotics. Due to the inefficiency and low efficacy of some antibiotics, researches with nanotechnology represent, possibly, new therapeutic strategies. Nanostructured drugs have presented some advantages over the conventional medicines, such as slow, gradual and controlled release, increased bioavailability, and reduced side effects, among others. In the study of Christ Vianna Santos et al. (2014), *in vitro* antimicrobial activity of tea tree oil nanoparticles against *Paenibacillus* species, including *P. larvae* and *M. plutonius* strains was evaluated. Tea tree oil registered MIC values of 0.18–6.25%, while the MIC values obtained for the tea tree oil nanoparticle were of 0.01–0.93%. The possible toxic effect of tea tree oil and tea tree oil nanoparticle has been assessed by the spraying application method in the concentrations higher than the MICs. Bee mortality was evident only in treatment with tea tree oil and the tea tree oil nanoparticles show no toxic effects after 7 days of observation (Christ Vianna Santos et al., 2014). Aćimović et al. (2017) and later Aćimović et al. (2018) in their research have showed very high antimicrobial activity of *Angelica archangelica* as well as essential oil of wild *Angelica* seeds. Kabir Mumu and Mahboob Hossain (2018) researched to determine tea tree oil abilities to control pathogenic bacteria: *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *P. vulgaris*, *A. hydrophila*, *E. coli*, *S. pneumoniae*, *B. subtilis*, *K. pneumonia* and *S. agalactiae*. After 24 hours incubation, tea tree oil showed minimum 96.94% against *E. coli* and maximum 100% inhibition against seven bacteria in this study. Tea tree oil showed some degree of antimicrobial properties with the highest activity index (1.6) against *S. agalactiae*. Tea tree oil exhibited observable zone against all the bacteria contrariwise, among nine antibiotics only two of them showed noticeable zone of inhibition to all the bacteria tested. According to study of Kabir Mumu and Mahboob Hossain (2018), tea tree oil has demonstrated remarkable antibacterial activity so it is expected that tea tree will gradually take place of conventional antibiotics to treat bacterial infection.

ANTIOXIDANT PROPERTIES OF TEA TREE

Research on bioactive principles of essential oils extracted from various herbs and spices has become increasingly popular because essential oils have been discovered to have many functional properties such as antimicrobial, antioxidant, and anticancer activities (Leal et al., 2003; Gulluce et al., 2003; Puvača et al., 2016). As a result, essential oils have been widely used as fumigants, cosmetics, and aroma therapeutic agents. Nowadays, many research groups are focusing their investigation in the pharmacological actions of essential oils from aromatic and medicinal plants (Puvača et al., 2013; Puvača et al., 2015; Kostadinović et al., 2015; Popović et al., 2016). Among them, tea tree (*Melaleuca alternifolia*) oil is one of the most important studied subjects because of its demonstrated wide spectrum of activities. Tea tree oil is mainly extracted by steam distillation from *Melaleuca alternifolia*, which, however, bears no resemblance to the taste or odor of the real tea, *Camellia sinensis*, Camelliaceae. Malenčić et al. (2018) in their research have showed dietary phenolics and antioxidant capacity of severls legumes seeds from the Central Balkans as well. Tea tree oil is composed of approximately 100 compounds, mainly monoterpenes, sesquiterpenes, and their alcohol derivatives, among which major components include terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, α-terpinolene, 1,8-cineole, etc. The international standard ISO 4730 requires commercial tea tree oil to have a minimum terpinen-4-ol content of 30% and a maximum 1,8-cineole content of 15%. However, most customers want to buy tea tree oil products containing the highest content of terpinen-4-ol and the lowest content of 1,8-cineole because it has been found that terpinen-4-ol has strong antimicrobial (Cox et al., 2001) and anti-inflammatory effects (Hart et al., 2000), while 1,8-cineole, also known as eucalyptol, is probably an allergen that is considered undesirable in tea tree oil products (Carson and Riley, 2001). Although the various functionalities, such as antibacterial, antifungal and anti-inflammatory of tea tree oil have been investigated in recent years (Brand et al., 2002), the antioxidant activity of tea tree oil and its bioactive components has not been reported. Therefore, it is interest to further explore the relationship between terpenic compounds and antioxidant activity in tea tree oil in order to provide a more complete characterization of its bio functional benefits. Also, additional knowledge of the antioxidant activity of tea tree oil may help to increase its market value. Many studies have shown that natural antioxidants in aromatic and medicinal plants are closely related with their bio functionalities, such as the reduction of chronic diseases such as DNA damage, mutagenesis, carcinogenesis (Zhu et al., 2002) and inhibition of growth of pathogenic bacteria (Giroux et al., 2001), which are often associated with the termination of free radical propagation in biological systems. Thus, antioxidant capacity is widely used as a parameter to characterize medicinal plants and their bioactive components. In study of Kim et al. (2004) the antioxidant activity of tea tree oil was investigated using two complimentary *in vitro* assays: the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay and the hexanal/hexanoic acid assay. Furthermore, some tea tree oil components showing strong antioxidant activity were separated from the crude tea tree oil by silica gel open column chromatography and C_{18}-high-pressure liquid chromatography and identified by gas chromatography-mass spectrometry. The antioxidant activities of tea tree oil and its bioactive components were compared with that of a commercial standard antioxidant, butylated hydroxytoluene. The antioxidant activity of four blend oils from limonene oil, lavender oil, pepper mint oil, eucalyptus oil, and tea tree oil was measured by the inhibition of 1, 1- diphenyl 2-picrylhyorazyl (DPPH), nitric oxide (NO), and reactive oxygen species (ROS) in the research of Park et al. (2017). All the oils showed about 90% DPPH inhibitory scavenging ability. Blending oils showed a decrease of 20% and 30% NO inhibitory scavenging ability at concentrations of 0.001 and 0.01‰, respectively. Three blending oils did not show a significant decrease of LPS-induced ROS generation. Tea tree oil and eucalyptus oil had higher activity on DPPH and NO than the corresponding other oils. Tea tree oil showed the strongest ROS activity among the oil products tested according the results of Park et al. (2017). The antioxidant activity of the samples seems to be based on their free radical scavenging capacity. The inhibitory activity was higher when the complex (blend) substance was treated than the single substance itself. Essential oils are complex mixtures isolated from aromatic plants which may possess antioxidant and anti-inflammatory activities of interest in the food and feed and could be useful tool for improving food quality (Džinić et al., 2015) equal as their powder forms (Spasevski et al., 2018). Review was done by Graca Miguel (2010), on the antioxidant and anti-inflammatory activities of several aromatic plants and essential oils, as well as survey of the methods generally used for the evaluation of antioxidant activity and some of the mechanisms involved in the anti-inflammatory activities of essential oils were also very precise reported.

ACARICIDAL PROPERTIES OF TEA TREE

Among ticks, *Ixodes ricinus* is one of the most efficient vectors of pathogenic agents such as *Borrelia burgdorferi* s.l., *Ehrlichia* (*Anaplasma phagocytophilum*) and *Rickettsia helvetica,* responsible respectively for Lyme disease, human granulocytic ehrlichiosis and a clinical condition with pathology similar to Mediterranean spotted fever. Although the hosts of preference of adult ticks are medium sized or large ungulates, their multi host behaviour gives them considerable opportunities to spread pathogens among numerous animal species, including humans, and to transmit infections that can be extremely serious. One means for combating the spread of these diseases more effectively is to reduce contact between humans and vector arthropods, not only by limiting visits, whether for work or recreational purposes, to environments at risk such as woods, etc., but above all by endeavouring to control the tick population (Potkonjak et al., 2017). The most widely used methods for controlling tick populations are based on the application of synthetic acaricides both in the environment and to animals. However, the indiscriminate use of these substances inevitably leads to resistance and potentially can harm the environment. There is a need to reduce the use of synthetic acaricides and to introduce alternative and supplementary methods for tick population control (Jurišić et al., 2010). A study of the literature shows that promising results have been achieved using both bacteria that are pathogenic for arthropods and extracts of certain plants. The essential oils of *Azadirachta indica* (neem tree oil) and of *Ocimum suave* have shown acaricidal and repellent properties against the larvae of *Amblyomma variegatum* and all stages of *Hyalomma anatolicum excavatum* and *Rhipicephalus appendiculatus* (Mwangi et al., 1995; Ndumu et al., 1999; Kaaya, 2000; Abdel-Shafy and Zayed, 2002). To the best of current knowledge, the antitick properties of the essential oil of *Melaleuca alternifolia*, a member of the *Myrtaceae* family, originating in Australia, or of other oils of the same genus (tea tree oils), have not been tested for tick control. These oils are known to possess bacteriostatic and germicidal properties and are used to cure infections of the skin and mucous membranes. Their apparent insecticidal and acaricidal properties have to date been tested on some human ectoparasites such as head lice and *Sarcoptes scabiei*, var. *hominis* (Walton et al., 2000; McCage et al., 2002). On the basis of previous findings Iori et al. (2005) designed a study to test the efficacy of tea tree oil against nymphs of *I. ricinus*. The aim of their study was to examine the acaricidal effect of essential oil of *Melaleuca alternifolia* (tea tree oil) at different doses (4, 6, 8 and 10 ml) and for different exposure times (30, 60, 90 and 120 min) on nymphs of *Ixodes ricinus*. A dose of 8 ml tea tree oil was lethal for more than 70% of ticks when inhaled and this effect was enhanced when the dose was increased to 10 ml (>80%). The effect was correlated with the duration of exposure of ticks to tea tree oil, with a significant effect being observed after 90 min exposure. In large scale ticks have bad influence also in cattle production. *Rhipicephalus australis* (formerly *Boophilus microplus*) is a one host tick responsible for major economic loss in tropical and subtropical cattle production enterprises. Control is largely dependent on the application of acaricides but resistance has developed to most currently registered chemical groups. Repellent compounds that prevent initial attachment of tick larvae offer a potential alternative to control with chemical toxicants. The repellent effects of *Melaleuca alternifolia* oil emulsions and two β-cyclodextrin complex formulations, a slow release form and a modified faster release form, were examined in a series of laboratory studies of Yim et al. (2016). In their research emulsions containing 4% and 5% tea tree oil applied to cattle hair in laboratory studies completely repelled ascending tick larvae for 24 h whereas 2% and 3% formulations provided 80% protection. At 48 h, 5% tea tree oil provided 78% repellence but lower concentrations repelled less than 60% of larvae. In a study conducted over 15 days, 3% tea tree oil emulsion applied to cattle hair provided close to 100% repellence for 2 days, but then protection fell to 23% by day 15. The faster realise formulation gave significantly greater repellence than the emulsion and the slow realise formulation from day 3 until the end of the study, providing almost complete repellence at day 3 (99.5%), then decreasing over the period of the study to 49% repellence at day 15. Proof of concept is established for the use of appropriately designed controlled release formulations to extend the period of repellence provided by tea tree oil against *R. australis* larvae. Lam et al. (2018) summarized possible effects of lowering the mite counts, relieving the demodex-related symptoms and modulating the immune system and especially the inflammatory response of tea tree oil and its extract terpinen-4-ol as an effective tool in moderating demodex-related diseases. Lam et al. (2018) in their research showed the typical treatments of tea tree oil and extract terpinen-4-ol in human demodicosis, their possible mechanism of actions, side effects and potential resistance in treating this condition. Although current treatments other than tea tree oil and extract terpinen-4-ol are relatively effective in controlling the demodex mite population and the related symptoms, more research on the efficacy and drug delivery technology is needed in order to assess its potential as an alternative treatment with minimal side effect profile, low toxicity and low risk of demodex resistance. The findings of Iori et al. (2005) show that tea tree oil has acaricidal properties and could be extremely useful in controlling ticks that are efficient vectors of pathogens.

CONCLUSIONS

Based on the summarized results and numerous findings of large number of researchers it could be with certainty concluded that *Melaleuca alternifolia* could be used as a powerful antimicrobial, antioxidant and acaricidal natural tool as an effective alternative to synthetic drugs, but the further in field investigation with this very worthy plant is more then necessary.

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