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In vitro Antimicrobial Activity of Commercially Available *Melaleuca alternifolia* (Tea Tree) Oil on Some Selected Clinical Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOE designed the study and wrote the first draft of the manuscript. Author HS wrote the protocol for the laboratory work and review the manuscript. Author JW carried out the laboratory work, including sourcing for the test organisms and data compilation. Author KEI managed the literature searches and review of manuscript. All authors read and approved the final manuscript

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ABSTRACT

Aim: To evaluate the *In vitro* antimicrobial activity of *Melaleuca alternifolia* oil (tea tree oil), (TTO) on selected clinical isolates of fungi and bacteria, including some multidrug resistant strains commonly associated with nosocomial infection.
Study Design: Laboratory experimental study.
Place and Duration of Study: Public Health England (PHE), Microbiology laboratory, Southampton University Hospital, England, May 2014.
Methodology: Three different concentrations (1%, 5% and 10%) of commercially available TTO

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were prepared (%v/v). Using an improvised disc diffusion antibiotic susceptibility testing method, the activity of TTO was tested against pure reference strains of *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922, clinical isolates of *Candida albicans*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes, Bacteroides fragilis*, methicillin- resistant *Staphylococcus aureus* (MRSA), extended spectrum beta lactamase *Escherichia coli* (ESBL) vancomycin-resistant *Enterococcus faecium* (VRE), and Carbapenemase-producing *Klebsiella pneumoniae* (CRKP). The zones of inhibition were measure for each organism at the various concentrations of TTO.

Results: All the organisms tested showed susceptibility to TTO at concentration range of 5% - 10%, except *Pseudomonas aeruginosa. Staphylococcus aureus* ATCC 25923, MRSA, and VRE did not show any susceptibility at TTO concentration of 1%. The zones of inhibition for the susceptible organisms ranged between 10mm-19mm at 5% concentration and 15 mm- 36 mm at 10% concentration of TTO.

Conclusion: TTO has a broad spectrum of *In vitro* activity against clinical pathogens. The essential oil or its active ingredient could be of potential benefit in the treatment of fungal, common bacterial and multidrug-resistant bacteria usually associated with hospital-acquired infections as well as mixed bacterial infections involving anaerobic bacteria.

Keywords: Melaleuca alternifolia; in vitro; antimicrobial activity; clinical pathogens.

1. INTRODUCTION

The essential oil of the plant *Melaleuca alternifolia,* referred to as Tea tree oil (TTO) is known historically to have profound medicinal value [1,2]. The plant *M. alternifolia* is native to Australia, and TTO which is extracted by steam distillation from the leaves and terminal branches of the plant is widely available over the counter in Australia, Europe, North America, and Africa as sole preparation or incorporated as the active ingredient in several topical preparations.

There is anecdotal evidence of the efficacy of TTO in the treatment of wound infection and several skin disorders, including acne and fungi infections such as athlete's foot, onychomycosis, and candidiasis [1,3-7]. In recent years, with the rising spate of antibiotic resistance, TTO has assumed some popularity in the medical literature. Some *in vitro* studies have demonstrated that TTO may have some antimicrobial activity against some multidrug-resistant bacteria such as methicillin- resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis,* and *Priopionibacterium* acnes [3,8,9]. Indeed, a randomized control trial demonstrated that TTO is efficacious in clearing MRSA colonization [10]. A range of yeast and dermatophyte species have also been shown to be susceptible to TTO at different concentrations [11,12].

The chemical composition and active ingredients of TTO has been analyzed. It contains largely of varieties of terpene hydrocarbon and oxygenated terpenes with terpenen-4-ol (fraction of the oxygenated terpene) as the main compound responsible for its antimicrobial activities [1]. Most standard commercial TTO contain 30-40% of terpenen-4-ol [13].

Despite some positive clinical outcome reported about TTO in the treatment of some known infections, there are very few and limited study on its *In vitro* activity, especially with regards to the stubborn multidrug- resistant bacteria. Also, the few available reports on its efficacy are not backed with information on the specific pathogens involved in the disease condition.

This study was undertaken to assess the *In vitro* antimicrobial property of TTO on selected clinical pathogens, including known multidrug resistant strains commonly associated with nosocomial infections. Data from this study may provide insight into possible indications for TTO in the clinical setting, or the use its active ingredient as a source and template for the synthesis of new antimicrobial drugs.

2. MATERIALS AND METHODS

2.1 Source and Preparation of TTO

Commercial TTO, distilled from the *Melaleuca alternifolia* leaves, (Tisserand, First Natural IP Ltd, England) was purchased over the counter in a pharmacy at Southampton, England. A sterile Olive oil, without antimicrobial activity, was also procured and used as a negative control and diluent for the TTO. The TTO and olive oil were pre-tested and validated for sterility by

inoculating blood agar and incubating for 24 hours. Three different concentrations (%v/v) of 1%, 5% and 10% of TTO were prepared using olive oil.

2.2 Microorganisms and Sources

In this experiment, pure reference strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, obtained from reference stock and clinical isolates obtained from freshly grown culture of clinical specimens were used. The clinical isolates tested included; a methicillin- resistant *Staphylococcus aureus* (MRSA) from a foot ulcer, extended spectrum beta lactamase *Escherichia coli* (ESBL) from blood culture, vancomycin - resistant *Enterococcus faecium* (VRE) from drainage of billary fistula, Carbapenem- resistant *Klebsiella pneumoniae* (CRKP) from blood culture, *Pseudomonas aeruginosa* from a surgical wound, *Streptococcus pyogenes* and *Bacteroides fragilis* both from inflamed tonsil, and *Candida albicans* from infected toe nail.

The identity of all the microorganisms were confirmed, after the initial biochemical testing, using MALDI-TOF MS (maltrix-assisted laser desorption/Ionization -time of flight mass spectrometry).

2.3 Antimicrobial Assay

All the organisms were initially screened against some relevant antibiotics to establish their susceptability and resistance pattern using the British Society of Antimicrobial Chemotherapy (BSAC) protocol [14]. As TTO is not a pharmaceutical drug, there is no standardized test for evaluating its antimicrobial activity. Therefore, in this experiment, the BSAC method for antimicrobial susceptibility testing was modified. Also, because TTO is lipophylic, dispersion in aqueous media results in a turbid suspension that makes determination of end point in susceptibility testing difficult, therefore the agar disc diffusion method was adopted for this study and implemented as follows;

The microorganisms were suspended in sterile saline water (trypton water for *Bactriodes* flagilis) and the turbidity was adjusted to 0.5% McFarland standard, equivalent to approximately $1x10^8$ CFU/ml. Using sterile swab sticks, the suspensions containing *Staphylococcus aureus* ATCC 25923, *Escherichia coli* 25922, MRSA, ESBL, CRKP, were streaked on standard Iso-Sensitest Agar (CM471 Thermo Fisher, Basingstoke, UK), while those containing *Streptococcus pyogenes,* VRE, and *Bacteroides fragilis*, were streaked on Mueller- Hinton sensitivity agar supplemented with sheep blood (CM337 Thermo Fisher, Basingstoke, UK). The suspension containing *Candida albicans* was streaked on Sabouraud Dextrose Agar (SDA). Four sterile discs of 5mm diameter, made from Whatman filter paper (England) with office hole punch were immediately placed on each plate at 40 mm apart, using sterile forceps. One of the four disc was placed at the center of each plate. Using a micropipette the centre disc was loaded with 10 µl of neat olive oil, while the other three had 10 µl drop of the different concentrations of TTO (1%, 5% and 10%) loaded directly onto them. All the plates were incubated at 37ºC in air, for 24hours, except for *Bacteroides fragilis* that was incubated in an anaerobic chamber for 48 hours. After incubation, the diameter of the zone of inhibition around each disc was measured in millimeters and recorded as antimicrobial activity of TTO.

2.4 Quality Control

The commercial TTO and olive oil were tested for sterility by culturing in blood agar for 24 hours before use. The sterile and non-germicidal olive oil disc serve as a blank and negative control for antimicrobial activity. Susceptible control strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were included to monitor test performance.

3. RESULTS

The susceptibility profile of the clinical isolates and the control strains of *Staphylococcus aureus* and *Escherichia coli* to a range of antibiotics commonly used in clinical practice is as shown below (Table 1). The zones of inhibition for the control strains were found to be within acceptable reference ranges [14]. The susceptibility profile of the various clinical isolates to the tested antibiotics also conformed considerably with their known pattern, except for *Pseudomonas aeruginosa* which showed sensitivity only to Meripenem and CRKP which demonstrated resistance to all the tested antibiotics.

Table 1. Antibiotic susceptibility pattern of microorganisms

Organism	Antibiotic Susceptibility																
	Amp	**Pip**	**Taz**	**Co-am**	**Cefu**	**Cefo**	**Cef**	**Cip**	**Gen**	**Mer**	**Ert**	**Chlo**	**Cot**	**Met**	**Ery**	**Van**	**Teic**
Staphylococcus aureus ATCC 25923	-	-	-	S	-	-	-	S	S	-	S	-	-	-	-	S	S
Staphylococcus. aureus (MRSA)	-	R	-	-	R	-	-	-	-	-	R	S	-	-	-	S	-
Escherichia coli ATCC 25922	-	-	-	R	-	S	S	S	S	S	-	-	-	-	R	-	-
Escherichia coli (ESBL)	R	-	-	S	R	R	R	R	-	S	S	R	R	-	-	-	-
Streptococcus pyogenes	S	-	-	S	-	-	-	-	-	-	S	S	-	S	S	-	-
Pseudomonas aeruginosa	R	-	-	R	R	R	R	R	-	S	R	-	-	-	-	-	-
Carbapenem-resistant *Klebsellia pneumoniae* (CRKP)	-	-	R	R	R	R	R	R	S	R	R	R	-	-	-	-	-
Vancomycin-resistant *Enterococcus faecium* (VRE)	R	R	-	R	-	-	-	-	R	-	-	-	-	-	R	R	R
Bacteroides fragilis	R	S	-	-	R	-	-	-	-	-	-	-	-	S	S	-	-
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: - = not tested, Amp=ampicillin, Pip=piperacillin, Taz=tazocin, Co-am=co-amoxiclav, Cefu=cefuroxime, Cefo=cefotaxime, Cef=ceftazidine, Cip=ciprofloxacin, Gen=gentamicin, Mer=meropenem, Ert= ertepenem, chl=chloramphenicol, Cot=cotrimoxazole, Met=metronidazole, Ery=erythromycin, Van=vancomycin, Teic= teicoplanin.

In the experiment with TTO (Table 2), all the organisms tested showed susceptibility to TTO at concentration range of 5% - 10%, except *Pseudomonas aeruginosa*. *Staphylococcus aureus* ATCC 25923, MRSA, and VRE did not show any susceptibility at TTO concentration of 1%. The zones of inhibition for the susceptible organisms ranged between 10 mm-19 mm at 5% concentration and 15 mm- 36 mm at 10% concentration of TTO. There was no zone of inhibition observed around any of the central disc loaded with neat olive oil (Fig. 1).

Generally, the zones of inhibition increased with the concentration of TTO. Also noted is the fact that both the susceptible (control) strains of *Staphylococcus aureus* and *Escherichia coli* and their corresponding resistant strains (MRSA and ESBL- *Escherichia coli*) showed remarkable susceptibility to TTO, although the differences in susceptibility were not statistically tested. *Bacteroides fragilis*, the only anaerobe tested and *Candida albicans*, the only yeast tested, also demonstrated remarkable sensitivity to TTO at the various concentrations.

4. DISCUSSION

Results from this study demonstrated that TTO has *In vitro* activity against a range of clinical pathogens, including the multidrug- resistant bacteria such as MRSA, ESBL, CRKP and VRE commonly associated with hospital-acquired infections. Interestingly, the antimicrobial activity exhibited by TTO in this experiment covered both Gram positive (*Staphylococcus aureus*, MRSA, *Streptococcus pyogenes*,) and Gram negative (*Escherichia coli*, *Bacteroides fragilis,* ESBL, CRKP, VRE) bacteria and fungi (*Candida albican*s).

Table 2. Susceptability of microorganisms to tea tree oil by zones of inhibition at various concentrations

Microorganism	Zone of inhibition (mm) at % v/v		
	1%	5%	10%
Staphylococcus aureus ATCC 25923	-	11	16
Staphylococcus. aureus (MRSA)	-	10	20
Escherichia coli ATCC 25922	9	19	26
Escherichia coli (ESBL)	8	14	22
Streptococcus pyogenes	11	13	15
Pseudomonas aeruginosa	-	-	-
Carbapenem- Resistant *Klebsiella pneumoniae*(CRKP)	12	17	18
Vancomycin-resistant Enterococcus faecium (VRE)	-	11	15
Bacteroides fragilis	9	16	36
Candida albicans	11	14	24

Key:- *No zone of inhibition was observed*

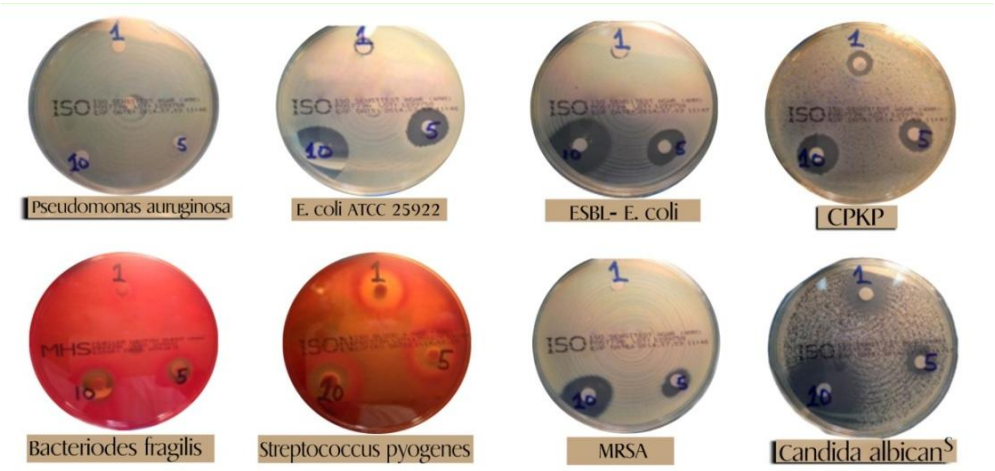

Fig. 1. Age plates showing zones inhibition of microorganisms by TTO

Although some investigators have reported the success of TTO in inhibiting the growth of MRSA and some of these pathogens [3,8,9,12,15], there is lack of data on the effect of TTO on most of the pathogens tested in this experiment. For instance, *Bacteroides fragilis*, a prototype anaerobic bacteria showed remarkable susceptibility to TTO. This finding is novel and suggestive of the potential use of TTO in the treatment of mixed bacterial infections involving anaerobic bacteria. Furthermore, ESBL *Escherichia coli*, VRE and CRKP are notorious superbugs with limited therapeutic options, and this study demonstrated that TTO has remarkable activity against these agents, and may be of potential therapeutic benefit.

Inouye et al. [16] reported the antimicrobial activity of vaporized TTO against some respiratory pathogens, including *Haemophilus influenza*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*, this study seems to substantiate this, as the Group A Streptococcus and *Bacteroides fragilis* strains tested were isolated from the inflamed tonsil of a five- year old child. Also, the fact that the MRSA and *Candida albicans* strains used in this experiment were isolated from the foot ulcer of a 29 -year old female and the infected toe nail of a 53 -year old female respectively, substantiate the anecdotal efficacy of TTO in the treatment of wounds and fungal infections of the nail.

Also observed in this study is that TTO did not show any activity against *Pseudomonas aeruginosa*. This contrast sharply with a previous study by Mickiene et al. [14], that reported a growth inhibition for *Pseudomonas aeruginosa* at 5% concentration of TTO. This discrepancy may be due to strain variation and the different compositions of TTO.

TTO at a lower concentration of 1% did not inhibit the growth of the VRE and *staphylococcus aureus* strains. This suggests that a higher concentration of TTO preparation may be required to initiate significant growth inhibition of these pathogens.

An important dimension to this study is that, the activity of TTO against the selected pathogens was tested in parallel with some conventional antibiotics, thus further substantiating the clinical potential of TTO in the treatment of infection caused by these multidrug- resistant pathogens. Nonetheless, this study was limited by the fact that only one isolate of the different organisms was tested. Therefore, no meaningful statistical conclusion could be drawn.

5. CONCLUSION

The preliminary data from this study suggests that TTO has a broad spectrum of *in vitro* activity against clinical pathogens. The essential oil or its active ingredient could be of potential benefit in the treatment of fungal, common bacterial and multidrug-resistant bacteria usually associated with hospital-acquired infections as well as mixed bacterial infections involving anaerobic bacteria. This however, would require more comprehensive studies and translational research in respect of its clinical efficacy, toxicity profile and the cost-effectiveness of any potential TTO treatment compared with available conventional antibiotic treatment.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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