Journal of Antimicrobial Chemotherapy (1998) **42**, 591–595

JAC

In-vitro activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp.

Katherine A. Hammer[a], Christine F. Carson[a] and Thomas V. Riley[a,b]

[a]Department of Microbiology, The University of Western Australia, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia, 6009; [b]The Western Australian Centre for Pathology and Medical Research, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia, 6009

The in-vitro activity of a range of essential oils, including tea tree oil, against the yeast candida was examined. Of the 24 essential oils tested by the agar dilution method against *Candida albicans* ATCC 10231, three did not inhibit *C. albicans* at the highest concentration tested, which was 2.0% (v/v) oil. Sandalwood oil had the lowest MIC, inhibiting *C. albicans* at 0.06%. *Melaleuca alternifolia* (tea tree) oil was investigated for activity against 81 *C. albicans* isolates and 33 non-*albicans Candida* isolates. By the broth microdilution method, the minimum concentration of oil inhibiting 90% of isolates for both *C. albicans* and non-*albicans Candida* species was 0.25% (v/v). The minimum concentration of oil killing 90% of isolates was 0.25% for *C. albicans* and 0.5% for non-*albicans Candida* species. Fifty-seven *Candida* isolates were tested for sensitivity to tea tree oil by the agar dilution method; the minimum concentration of oil inhibiting 90% of isolates was 0.5%. Tests on three intra-vaginal tea tree oil products showed these products to have MICs and minimum fungicidal concentrations comparable to those of non-formulated tea tree oil, indicating that the tea tree oil contained in these products has retained its anticandidal activity. These data indicate that some essential oils are active against *Candida* spp., suggesting that they may be useful in the topical treatment of superficial candida infections.

Introduction

Many essential oils have been advocated for use in complementary medicine for bacterial and fungal infections including boils, acne, gingivitis and vaginal candidiasis.[1–4] However, few of the many claims of therapeutic efficacy have been validated adequately by either in-vitro testing or in-vivo clinical trials. Unless these claims have been substantiated scientifically, complementary medicines are unlikely to secure a place in conventional healthcare. One essential oil for which some data exist is tea tree oil. This oil is steam-distilled from leaves of the Australian native plant *Melaleuca alternifolia* and has been used widely as a topical antiseptic in Australia for almost 80 years.[5] Recent investigations in Australia have confirmed that tea tree oil has in-vitro activity against a wide range of Gram-positive and Gram-negative bacteria, including *Propioni - bacterium acnes* and methicillin-resistant *Staphylococcus aureus*.[6–8]

The overgrowth of *Candida* spp. in the vagina is termed vaginal candidiasis, or more commonly, thrush.[9] The normal vaginal flora is dominated by *Lactobacillus* spp. which acidify the environment and prevent pathogenic organisms from colonizing the vagina and causing disease.[10] Many factors, including antibiotics, oral contraceptives, infection and dietary changes, can alter vaginal flora, providing opportunity for the overgrowth of *Candida* spp.[4,10,11] Approximately 80–90% of thrush cases are caused by *Candida albicans* with *Candida glabrata, Candida tropicalis, Candida parapsilosis* and others being responsible for the remainder.[11]

Essential oils have been recommended for use as home remedies for the treatment of vaginal candidiasis by numerous books and articles in the popular press.[4,12] Products containing essential oils, designed for the treatment of vaginal conditions and formulated specifically for intra-vaginal use, are available in the UK, USA, Australia and Europe.[13,14] As yet, no data have been published as to the in-vitro or in-vivo efficacy of such products containing tea tree oil.

The present investigation examines the in-vitro susceptibility of *Candida* spp. to a range of essential oils, including tea tree oil and intra-vaginal tea tree oil products.

Materials and methods

Organisms

One hundred and fourteen *Candida* spp. isolates were obtained from the Department of Microbiology at The University of Western Australia, and the Infection Control Unit and Bacteriology and Mycology Section of The Western Australian Centre for Pathology and Medical Research. Reference isolates consisted of *C. albicans* ATCC 10231, ATCC 90028, ATCC 90029, *C. parapsilosis* ATCC 90018, *S. aureus* NCTC 6571 and *Escherichia coli* NCTC 10418. The clinical isolates comprised *C. albicans* (n = 78), *C. glabrata* (n = 12), *Candida guilliermondii* (n = 1), *C. parapsilosis* (n = 13), *Candida pseudotropicalis* (n = 1), *C. tropicalis* (n = 4), and *Candida stellatoidea* (n = 1). Clinical isolates were identified by the germ tube test[15] and an ID32C strip (bioMérieux Vitek, Inc., Hazelwood, MO, USA) for non-*albicans* isolates.

Essential oils and intra-vaginal tea tree oil products

All essential oils except tea tree oil were kindly provided by Sunspirit Oils Pty. Ltd, Byron Bay, NSW, Australia. Tea tree oil (batch 93/04) was kindly supplied by Australian Plantations Pty. Ltd, Wyrallah, NSW, Australia. The tea tree oil complied with the International Standard ISO 4730 and the concentrations of terpinen-4-ol and 1,8-cineole, as determined by gas chromatography–mass spectrometry, were 37.1% and 3.2%, respectively.

Intra-vaginal tea tree oil products were provided by Australian Bodycare Corporation Pty. Ltd, Currumbin, QLD, Australia and Phytopharmaceutical Products Pty. Ltd, Sydney, NSW, Australia.

Inoculum preparation

Each isolate was inoculated into Mueller–Hinton broth (MHB) (Unipath Ltd, Basingstoke, UK) which was then incubated overnight at 35°C with shaking. Overnight cultures were diluted to give final inocula for agar dilutions of approximately 10^3 and 10^4 cfu/spot, for *Candida* spp. and bacteria, respectively. The final inoculum for broth microdilution and product evaluation tests was approximately 5×10^5 cfu/mL for all organisms. Viable counts were performed to confirm the inoculum size for broth and product tests.

Agar dilution method

Agar plates were prepared by adding 0.5% (v/v) Tween 20 (Sigma Chemical Co., St Louis, MO, USA) to molten Mueller–Hinton agar (MHA) (Unipath), and then adding the essential oil in doubling dilutions ranging from 2% to 0.03% (v/v). Control plates of MHA with Tween 20 but no oil and a blood agar (Unipath) plate were included. Plates were dried for 30 min and then inoculated with each isolate in duplicate using a multipoint replicator (Mast Laboratories Ltd, Liverpool, UK). Plates were incubated aerobically at 35°C, immediately after inoculating. MICs were determined at 24 h for bacteria and 48 h for *Candida* spp. The MIC was defined as the lowest concentration of oil inhibiting visible growth. The presence of one or two colonies was disregarded. The minimum concentration of oil which inhibited at least 90% of the isolates tested was defined as the MIC_{90}.

C. albicans ATCC 10231 was used for tests against the 24 essential oils. *S. aureus* and *E. coli* were used as control strains when testing *Candida* spp. isolates against tea tree oil by the agar dilution method.

Broth microdilution method

A range of doubling dilutions of tea tree oil from 2% to 0.03% (v/v) was prepared in MHB in a 96-well microtitre tray. Tween 80 (Sigma) was included at a final concentration of 0.001% (v/v) to enhance oil solubility. After inoculation and incubation at 35°C for 48 h, subcultures of 10 μL were taken from each well and spot inoculated on to MHA. Subcultures were incubated aerobically and MICs and minimum fungicidal concentrations (MFCs) determined. The MIC was defined as the lowest concentration of oil resulting in the maintenance or reduction of the inoculum, and the MFC as the lowest concentration of oil resulting in the death of 99.9% of the inoculum. The MIC_{90} was determined as described in the agar dilution method, while the MFC_{90} was defined as the concentration of tea tree oil fungicidal for at least 90% of the isolates tested. Isolates were tested in duplicate and were retested if resultant MIC or MFC values differed. *C. albicans* ATCC 10231 was used in all broth microdilution tests as a control.

In-vitro testing of tea tree oil products

Tea tree oil products were diluted in sterile distilled water so that after inoculation, the final concentrations of tea tree oil ranged from 2.0% to 0.03% (w/v). Each dilution was inoculated with the test organism, *C. albicans* ATCC 10231, in double strength MHB to a final concentration of approximately 5×10^5 cfu/mL. After incubation at 35°C for 24 h, 100 μL samples were taken, diluted one in ten and spread on to blood agar. These subcultures were incubated at 35°C for 24–48 h, then MICs and MFCs were determined as described in the section above on the 'Broth microdilution method'.

Results

The MICs obtained for the 24 essential oils tested are shown in Table I. No oil inhibited *C. albicans* ATCC 10231 at the lowest concentration tested, which was 0.03%. All

Table I. MICs of 24 essential oils tested against *C. albicans* ATCC 10231 using the agar dilution method

Common name	Botanical name	MIC (%v/v)
Sandalwood	*Santalum album*	0.06
Lemongrass	*Cymbopogon citratus*	0.12
Spearmint	*Mentha spicata*	0.12
Oregano	*Origanum vulgare*	0.12
Bay	*Pimenta racemosa*	0.12
Clove	*Syzigium aromaticum*	0.12
Petitgrain	*Citrus aurantium*	0.25
Coriander	*Coriandrum sativum*	0.25
Citronella	*Cymbopogon nardus*	0.25
Tasmanian lavender	*Lavandula angustifolia*	0.25
French lavender	*Lavandula angustifolia*	0.5
Peppermint	*Mentha piperita*	0.5
Basil	*Ocimum basilicum*	0.5
Sage	*Salvia officinalis*	0.5
Celery	*Apium graveolens*	1.0
Frankincense	*Boswellia carteri*	1.0
Ylang ylang	*Cananga odorata*	1.0
Bergamot	*Citrus bergamia*	1.0
Eucalyptus	*Eucalyptus fruticetorum*	1.0
Lemon	*Citrus limon*	2.0
Juniper	*Juniperus communis*	2.0
Cedarwood	*Cedrus atlantica*	>2.0
Evening primrose	*Oenothera biennis*	>2.0
Sweet almond	*Prunus dulcis*	>2.0

Table III. MICs and MFCs of tea tree oil products against *C. albicans* ATCC 10231

Product (% tea tree oil)	MIC (%w/v)	MFC (%w/v)
Gel (3%)	0.25	0.5
Gel (10%)	0.12	0.25
Pessary (10%)	0.25	0.5

except cedarwood, sweet almond and evening primrose inhibited *C. albicans* ATCC 10231 at concentrations of ⩽2.0%.

MIC and MFC data for tea tree oil, obtained by the agar and broth dilution methods are given in Table II. Using agar dilution, the MIC_{90} was ⩽0.5%, for all *Candida* species and strains tested, while MICs for the control isolates *S. aureus* and *E. coli* were 0.5% and 0.25%, respectively. Using broth microdilution, both the MIC_{90} and MFC_{90} for *C. albicans* were 0.25%. The MIC_{90} and MFC_{90} for *C. parapsilosis* and *C. glabrata* were 0.25% and 0.5%, respectively. The MIC and MFC of tea tree oil against the control isolate *C. albicans* ATCC 10231 were both 0.25%.

Results for the testing of tea tree oil products are shown in Table III. All products showed MICs and MFCs similar to non-formulated tea tree oil.

Discussion

There are data available referring to the anticandidal activity of the more familiar essential oils such as peppermint and thyme.[16–19] Some oils, such as frankincense

Table II. Cumulative MICs and MFCs for *Candida* spp. tested by agar dilution (n = 57) and broth microdilution (n = 114) methods

			Tea tree oil concentration (%v/v)		
Organism	Method	Parameter	0.12	0.25	0.5
C. albicans	agar	MIC	0	**21**[a]	24
	broth	MIC	48	**81**	81
	broth	MFC	2	**81**	81
C. glabrata	agar	MIC	1	**12**	12
	broth	MIC	4	**11**	12
	broth	MFC	1	4	**12**
C. parapsilosis	agar	MIC	0	**13**	14
	broth	MIC	9	**14**	14
	broth	MFC	0	11	**14**
Other *Candida* spp.[b]	agar	MIC	1	5	7
	broth	MIC	5	7	7
	broth	MFC	2	7	7

[a] MIC_{90} and MFC_{90} values are in bold type.
[b] *C. guilliermondii* (n = 1), *C. pseudotropicalis* (n = 1), *C. tropicalis* (n = 4), *C. stellatoidea* (n = 1).

and evening primrose, appear not to have been tested previously against *Candida* spp. Comparison of our data with anticandidal data reported previously showed most results to be similar.[16–19] The data obtained in this study show that essential oils such as sage and sandalwood, which have been suggested for use in antiseptic or antimicrobial capacities,[12,16] possess anticandidal properties *in vitro*.

There are difficulties with comparing published results of the antimicrobial activity of some essential oils. These arise where the common name but not the botanical name is specified and where no data are given about the chemical composition of the oil. Also, there are many different methods used to investigate antimicrobial activity and the results obtained by these methods are not always directly comparable. These reasons may, in part, explain the differences in results obtained by different research groups.

Tea tree oil has activity against a wide range of Gram-positive and Gram-negative bacteria.[8] Less information is available about the spectrum of activity and mode of action against *Candida* spp. Previous reports have indicated MICs of 0.04%,[16] 0.2%[20] and 0.44%[21] for *C. albicans*, and 0.22% for *Candida* spp.[21] Our data support the above-mentioned reports and indicate that *Candida* spp. are susceptible to concentrations of ⩽0.5% tea tree oil. In addition, there was little inter-species variation in susceptibility and all *Candida* spp. tested were uniformly susceptible.

Several products utilizing tea tree oil as an active ingredient have been developed for the treatment of vaginal candidiasis. These include intra-vaginal pessaries, gels and douche preparations. Products such as these have been available for many years[13,14,22] and offer alternatives to the more conventional therapies of azole derivatives, which are available as intra-vaginal creams or pessaries, or are taken orally.[23]

The anticandidal activity demonstrated by the products tested in this study indicates that formulated tea tree oil appears to retain the activity of non-formulated tea tree oil. Demonstrating that products were active *in vitro* suggests that these products are potentially useful *in vivo*. However, in-vivo effectiveness can only be determined with a randomized clinical trial to evaluate each product thoroughly. It is also possible that product ingredients other than the tea tree oil, may have anti-candida activity, and are contributing to the total activity of the product. However, this is unlikely as the tea tree oil MICs were the same for pure tea tree oil and for product. To determine accurately any synergic or antagonistic effect of product excipients it will be necessary to perform chequerboard analyses.

Ideally, a vaginal candidiasis treatment would effect a mycological cure as well as relieve the candidiasis-associated symptoms of vulval and vaginal pruritis, vulval erythema and discharge. There are isolated case reports suggesting that tea tree oil products may assist in relieving the symptoms of pruritis and vaginal 'burning'.[13,24] These preliminary claims are promising and future investigations may provide support for these observations.

One issue that may require further investigation is that of adverse reactions to tea tree oil. Peña[22] investigated the use of intravaginal tea tree oil preparations for a variety of vaginal conditions and found no vaginal irritation in 130 trial participants. Similarly, a study by Barnes[13] found no vaginal irritation in 20 trial participants. The incidence of adverse reactions to tea tree oil in the general population is as yet unknown, but these studies suggest that the incidence of vaginal irritation may be relatively low.

In summary, tea tree oil shows significant promise as a potential therapeutic agent for the treatment of vaginal candidiasis. In-vitro results indicate susceptibility at low concentrations (⩽0.5% v/v) and products containing tea tree oil maintained in-vitro efficacy. What is now required is clinical trials to determine the usefulness of tea tree oil *in vivo*.

Acknowledgements

The assistance of the Division of Microbiology and Infectious Diseases, The Western Australian Centre for Pathology and Medical Research, Nedlands, in obtaining clinical isolates is appreciated. This work was supported by Australian Bodycare Corporation Pty. Ltd, Currumbin, QLD, Australia and, in part, by a grant from the Rural Industries Research and Development Corporation (UWA-24A).

References

1. Stanway, A. (1982). *Alternative Medicine: A Guide to Natural Therapies*. Penguin Books Ltd, Harmondsworth.

2. Hoffman, D. L. (1987). *The Herb User's Guide*. Thorsons Publishing Group, Wellingborough, Northamptonshire.

3. Tyler, V. E. (1993). *The Honest Herbal*, 3rd edn. Haworth Press Inc., Binghampton, NY.

4. Marti, J. (1995). *The Alternative Health and Medicine Encyclopedia*. Gale Research International Inc., Detroit, MI.

5. Altman, P. M. (1988). Australian tea tree oil. *Australian Journal of Pharmacy* **69**, 276–8.

6. Carson, C. F. & Riley, T. V. (1994). Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia. Letters in Applied Microbiology* **19**, 24–5.

7. Carson, C. F., Cookson, B. D., Farrelly, H. D. & Riley, T. V. (1995). Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia. Journal of Antimicrobial Chemotherapy* **35**, 421–4.

8. Hammer, K. A., Carson, C. F. & Riley, T. V. (1996). Susceptibility of transient and commensal skin flora to the essential oil of *Melaleuca alternifolia* (tea tree oil). *American Journal of Infection Control* **24**, 186–9.

9. Nyirjesy, P., Seeney, S. M., Grody, M. H. T., Jordan, C. A. & Buckley, H. R. (1995). Chronic fungal vaginitis: the value of cultures. *American Journal of Obstetrics and Gynecology* **173**, 820–3.

10. Mårdh, P.-A. (1991). The vaginal ecosystem. *American Journal of Obstetrics and Gynecology* **165**, 1163–8.

11. Horowitz, B. J., Giaquinta, D. & Ito, S. (1992). Evolving pathogens in vulvovaginal candidiasis: implications for patient care. *Journal of Clinical Pharmacology* **32**, 248–55.

12. Newall, C. A., Anderson, L. A. & Phillipson, J. D. (1996). *Herbal Medicines. A Guide for Health-care Professionals*. The Pharmaceutical Press, London.

13. Barnes, B. (1989). The development of topical applications containing tea tree oil for vaginal conditions. In *Modern Phytotherapy—The Clinical Significance of Tea Tree Oil and Other Essential Oils. Proceedings of a Conference in Sydney, September 17 1989*, Vol. I, pp. 27–35. Australian Tea Tree Industry Association, Coraki, Australia.

14. Blackwell, A. L. (1991). Tea tree oil and anaerobic (bacterial) vaginosis. *Lancet* **337**, 300.

15. Crissey, J. T., Lang, H. & Parish, L. C. (1995). *Manual of Medical Mycology*. Blackwell Science, Cambridge, MA.

16. Beylier, M. F. (1979). Bacteriostatic activity of some Australian essential oils. *Perfumer and Flavorist* **4**, 23–5.

17. Morris, J. A., Khettry, A. & Seitz, E. W. (1979). Antimicrobial activity of aroma chemicals and essential oils. *Journal of the American Oil Chemists' Society* **56**, 595–603.

18. Yousef, R. T. & Tawil, G. G. (1980). Antimicrobial activity of volatile oils. *Pharmazie* **35**, 698–701.

19. Hili, P., Evans, C. S. & Veness, R. G. (1997). Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. *Letters in Applied Microbiology* **24**, 269–75.

20. Southwell, I. A., Hayes, A. J., Markham, J. & Leach, D. N. (1993). The search for optimally bioactive Australian tea tree oil. *Acta Horticulturae* **344**, 256–65.

21. Nenoff, P., Haustein, U. F. & Brandt, W. (1996). Antifungal activity of the essential oil of *Melaleuca alternifolia* (tea tree oil) against pathogenic fungi in vitro. *Skin Pharmacology* **9**, 388–94.

22. Peña, E. F. (1962). *Melaleuca alternifolia* oil. Its use for trichomonal vaginitis and other vaginal infections. *Obstetrics and Gynecology* **19**, 793–5.

23. Working Group of the British Society for Medical Mycology. (1995). Management of genital candidiasis. *British Medical Journal* **310**, 1241–4.

24. Merkur, H. (1989). The impact of HPV (human papilloma virus) in gynaecology. In *Modern Phytotherapy – The Clinical Significance of Tea Tree Oil and Other Essential Oils. Proceedings of a Conference in Sydney, September 17 1989*. Vol. I, pp. 36–41. Australian Tea Tree Industry Association, Coraki, Australia.

Received 9 February 1998; accepted 27 May 1998