The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials.

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ORIGINAL ARTICLE

The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials

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antibiotic, essential oils, *Melaleuca alternifolia*, *Mentha piperita*, *Rosmarinus officinalis*, synergistic; additive; antagonistic; combination, *Thymus vulgaris*.

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Abstract

Aims: Due to the emergence of multi-drug resistance, alternatives to conventional antimicrobial therapy are needed. This study aims to investigate the *in vitro* pharmacological interactions between essential oils (considered valuable as natural therapeutic treatments) and conventional antimicrobials (ciprofloxacin/amphotericin B) when used in combination.

Methods and Results: Interactions of the essential oils (*Melaleuca alternifolia*, *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis*) when combined with ciprofloxacin against *Staphylococcus aureus* indicate mainly antagonistic profiles. When tested against *Klebsiella pneumoniae* the isobolograms show antagonistic, synergistic and additive interactions depending on the combined ratio. The *R. officinalis*/ciprofloxacin combination against *K. pneumoniae* displayed the most favourable synergistic pattern. The interactions of *M. alternifolia* (tea tree), *T. vulgaris* (thyme), *M. piperita* (peppermint) and *R. officinalis* (rosemary) essential oils with amphotericin B indicate mainly antagonistic profiles when tested against *Candida albicans*.

Conclusion: While a number of interactions show complete antagonism, others show varied (synergistic, additive and/or antagonistic) interactions, thus the efficacy is dependent on the ratio in which the two components co-exist.

Significance and Impact of the Study: The predominant antagonistic interactions noted here, suggests that some natural therapies containing essential oils should be used with caution when combined with antibiotics.

Introduction

The emergence of multi-drug resistant strains because of the incorrect and overuse of existing antimicrobials is becoming a formidable threat in the fight against disease. Thus, alternatives to the standard treatments with single agents are presently being sought. In clinical practice, combination antibiotic therapy is being used in an attempt to broaden the bacterial spectrum and to avoid the emergence of resistant strains (Lambert 2000). Furthermore, to avoid the undesirable toxic effects of antimicrobial therapy, the combination with herbal products may be an innovative alternative to prescriptive treatment protocols (Harris 2002). The antimicrobial properties of essential oils have been known for many years (Williams *et al.* 1998) and those from popular commercially available aromatic plants such as *Melaleuca alternifolia* (tea tree), *Thymus vulgaris* (thyme), *Mentha piperita* (peppermint) and *Rosmarinus officinalis* (rosemary) have been used extensively to treat bacterial and fungal infections (Filoche *et al.* 2005). Potential synergy of essential oils with antibiotics has previously been postulated, with the aim to alleviate the burden of antimicrobial resistance to conventional antimicrobials. In a study conducted by Shin and Kang (2003), an essential oil fraction of *Agastache rugosa* combined with ketoconazole was found to be

synergistic against *Blastischizomyces capitatus*. Further research on *Aspergillus* species demonstrated additive effects of *Pelargonium graveolens* (rose geranium) fractions with amphotericin B and ketoconazole (Shin 2003). Recent research on *P. graveolens* oil, in combination with norfloxacin demonstrated synergy when tested against *Staphylococcus aureus* and *Bacillus cereus* (Rosato *et al.* 2007). Combinations of *Allium sativum* (garlic) with ketoconazole were synergistic when investigated against three species of *Trichophyton* (Pyun and Shin 2006). Dimitrijević *et al.* (2007) demonstrated that lower concentrations of oil combinations (*T. vulgaris* and *R. officinalis* with the preservative lactic acid) were more effective than higher concentrations. These studies are based on the common assumption that the combination of an essential oil with a conventional antimicrobial would produce a synergistic profile. An assumption derived from the understanding that the antibiotic and essential oil constituents attack the microbe at different sites and/or that the essential oil components enhance skin permeation in transdermal drug delivery (Harris 2002). With this in mind, the *in vitro* pharmacological interactions between a selection of popular commercial oils (*M. alternifolia*, *T. vulgaris*, *M. piperita* and *R. officinalis*) and conventional antimicrobials (ciprofloxacin or amphotericin B) were investigated to determine if similar synergistic interactions might occur.

Materials and methods

Source of materials

Commercial essential oils (*M. alternifolia, T. vulgaris, M. piperita* and *R. officinalis*) and their GC–MS data were obtained from a flavour and fragrance company in France. Quantification of major compounds was confirmed by GC. The major compounds and their relative percentages for these oils have previously been reported (van Vuuren and Viljoen 2006). Antimicrobials (ciprofloxacin and amphotericin B) were obtained from Sigma-Aldrich. The selection of allopathic antimicrobials ciprofloxacin and amphotericin B is based on previous studies where in various combinations, synergistic interactions were noted (Critchley *et al.* 2003; Wang *et al.* 2003; Johnson *et al.* 2004). Microbial cultures *S. aureus* (ATTC 25923), *K. pneumoniae* (NCTC 9633) and *Candida albicans* (ATCC 10231) were selected for this study on the basis of their importance in frequently occurring nosocomial infections (Giamarellou 2002; Borghesi and Stronati 2008). All stock cultures were obtained from the National Health Laboratory Services, South Africa with the exception of *C. albicans*, which was obtained from the South African Bureau of Standards.

Antimicrobial interactions

The essential oils (*M. alternifolia*, *T. vulgaris*, *M. piperita* and *R. officinalis*) at starting stock concentrations of 128 mg ml^{-1} in acetone were combined with ciprofloxacin at a starting stock concentration of 0·01 mg ml^{-1} in nine different ratios i.e. 9 : 1; 8 : 2; 7 : 3; 6 : 4; 5 : 5; 4 : 6; 3 : 7; 2 : 8 and 1 : 9. The microdilution MIC method was adapted from Eloff (1998) and determined for the essential oil and the antimicrobial independently (Table 1) and for all ratios (Figs 1–4). The NCCLS (2003) guidelines were used to ensure that accurate microbiological assay and transfer techniques were followed. Positive bactericidal controls i.e. ciprofloxacin and amphotericin B were included in each assay to confirm the antimicrobial susceptibility. Serial dilutions were performed on stock solutions and overnight bacterial cultures (100 μl), diluted in fresh Tryptone Soya broth at a 1 : 100 ratio, yielding an approximate inoculum size of 1×10^6 CFU ml^{-1} was added to all the wells. Similarly, the essential oils were combined with amphotericin B at a starting stock concentration of 0·1 mg ml^{-1} and tested against *C. albicans*. The stock concentrations of antimicrobial (ciprofloxacin and amphotericin B) and essential oil were selected on the basis of having obtainable end point MIC values, as determined in previous studies (van Vuuren and Viljoen 2006). Optimal incubation conditions were followed (37°C for 24 h for bacterial strains and 48 h for the yeast). The study was undertaken in triplicate. The mean MIC values were plotted on isobolograms (Figs 1–4) using Graphpad Prism® software, allowing for a graphical representation of the interaction of the

Table 1 The mean MIC values (mg ml^{-1}) of essential oil and conventional antimicrobial against three pathogens

	Pathogen		
Test substance	*Staphylococcus aureus* ATTC 25923	*Klebsiella pneumoniae* NCTC 9633	*Candida albicans* ATCC 10231
Rosmarinus officinalis (rosemary)	6·0	8·0	6·0
Melaleuca alternifolia (tea tree)	6·7	6·0	6·0
Thymus vulgaris (thyme)	4·7	4·0	3·3
Mentha piperita (peppermint)	8·0	8·0	6·0
Conventional antimicrobial	8·1 E-04*	3·3 E-04*	1·7 E-02†

*Ciprofloxacin.
†Amphotericin B.

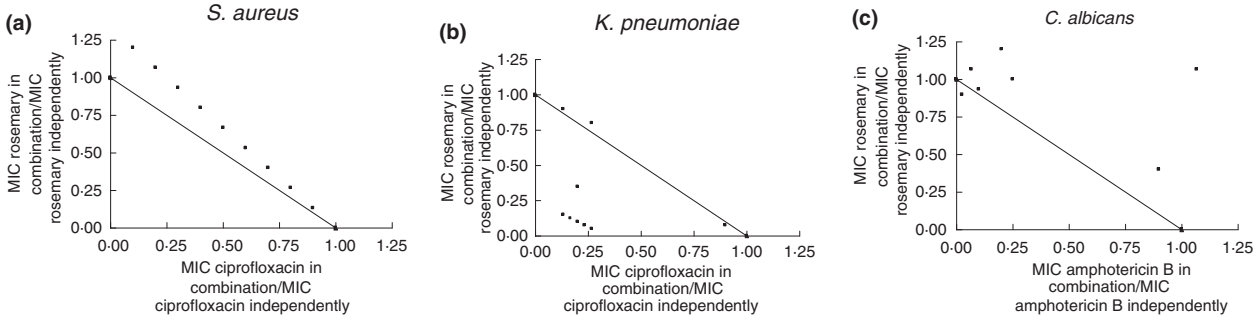

Figure 1 The combination profiles of *Rosmarinus officinalis* (rosemary) essential oil with commercial antimicrobials.

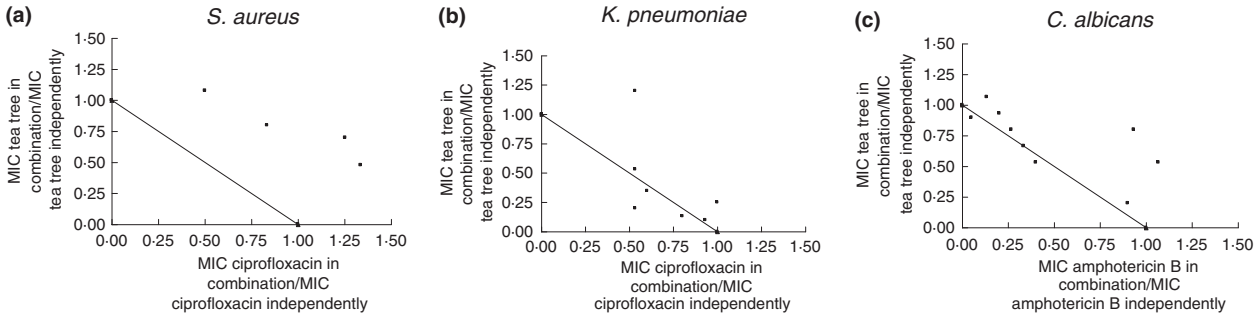

Figure 2 The combination profiles of *Melaleuca alternifolia* (tea tree) essential oil with commercial antimicrobials.

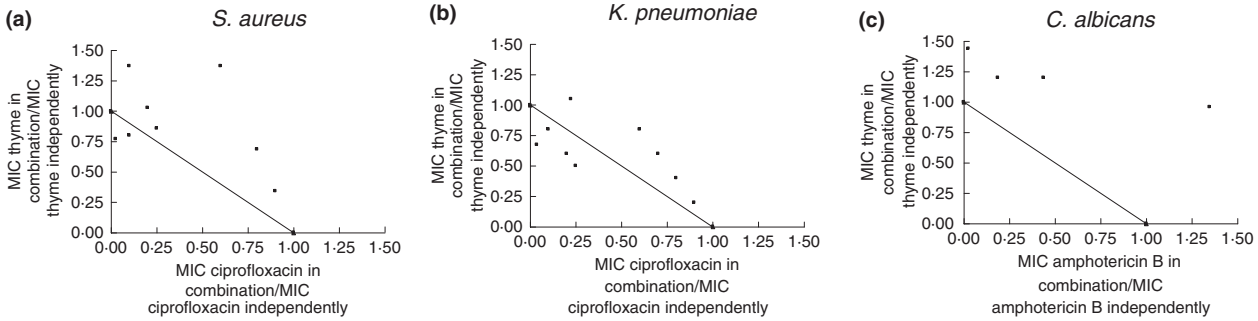

Figure 3 The combination profiles of *Thymus vulgaris* (thyme) essential oil with commercial antimicrobials.

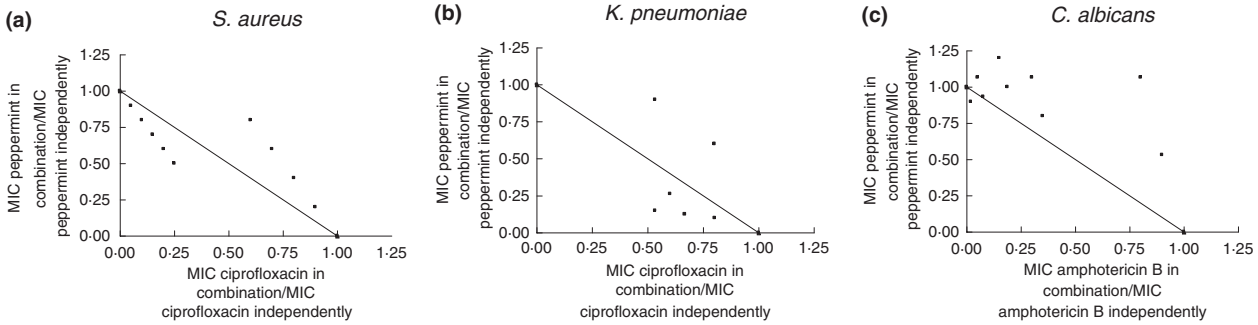

Figure 4 The combination profiles of *Mentha piperita* (peppermint) essential oil with commercial antimicrobials.

various combinations (Stergiopoulou *et al.* 2008). Isobologram ratios were calculated on the basis of the following equations:

$$X = \frac{\text{MIC value of combined essential oil and antimicrobial}}{\text{MIC value of antimicrobial alone}}$$

$$Y = \frac{\text{MIC value of combined essential oil and antimicrobial}}{\text{MIC value of essential oil alone}}$$

The isobologram can be interpreted by examining the data points of the ratios where the MIC for each concentration is determined in relation to the independent MICs (shown as a straight line) and extrapolating synergy (below the line), antagonism (above the line) and additive in the vicinity closest to or on the line (Berenbaum 1978). Data from which the isobolograms were constructed are presented as fractional inhibitory concentrations (FICs). The FIC index (Table 2) was calculated on the basis of the above mentioned equation where $\sum\text{FIC} = X + Y$ and interactions defined as; FIC < 1 = synergy, FIC > 1 = antagonism and where the FIC value = 1 suggests additive interactions (Berenbaum 1978).

Results

The MIC values for the essential oils and antimicrobials are presented in Table 1. Independently, the essential oils displayed moderate antimicrobial activity with MIC values in the range (3·3–8·0 mg ml^{-1}).

The combination profiles for *R. officinalis* (rosemary) essential oil ratios with the commercial antimicrobial ciprofloxacin or amphotericin B is depicted in Fig. 1 and Table 2. Studies with *S. aureus* and *C. albicans* show a predominantly antagonistic profile. Combinations with *K. pneumoniae* indicated either an additive (two ratios) or synergistic (seven ratios) effect. Synergy is best noted for ratios 9 : 1 (FIC 0·97), 8 : 2 (FIC 0·32), 7 : 3 (FIC 0·31), 6 : 4 (FIC 0·30), 5 : 5 (FIC 0·29), 4 : 6 (FIC 0·28) and 3 : 7 (FIC 0·55) in Table 2.

The combination profiles of *M. alternifolia* (tea tree) essential oil with the commercial antimicrobials are presented in Fig. 2 and Table 2. When *M. alternifolia* oil is combined with ciprofloxacin and tested against *S. aureus*, antagonism was noted for all ratios. This is especially interesting as tea tree oil is often used as an antiseptic to treat skin infections (Shemesh and Mayo 1991). Furthermore, tea tree oil is commonly used as an effective topical treatment against Staphylococcal infections (Halcón and Milkus 2004). Five antagonistic ratios for *S. aureus* (ratios 9 : 1, 8 : 2, 7 : 3, 6 : 4 and 5 : 5) and three antagonistic ratios (9 : 1, 8 : 2 and 1 : 9) for *K. pneumoniae* were not plotted on the isobologram as the points were out of the

Table 2 The $\sum$FIC index of essential oil in combination with the conventional antimicrobial where FIC < 1 = synergy (indicated in bold), FIC > 1 = antagonism and where the FIC value = 1 suggests additive interactions

Ratio		*Staphylococcus aureus* ATTC 25923				*Klebsiella pneumoniae* NCTC 9633				*Candida albicans* ATCC 10231			
Antimicrobial*	Oil	*R. officinalis*†	*M. alternifolia*‡	*T. vulgaris*§	*M. piperita*¶	*R. officinalis*†	*M. alternifolia*‡	*T. vulgaris*§	*M. piperita*¶	*R. officinalis*†	*M. alternifolia*‡	*T. vulgaris*§	*M. piperita*¶
9	1	1·03	7·70	1·24	1·10	**0·97**	1·85	1·10	1·84	1·30	1·10	2·31	1·43
8	2	1·07	7·07	1·49	1·20	**0·32**	1·70	1·20	2·24	2·13	1·60	2·60	1·87
7	3	1·10	3·22	2·59	1·30	**0·31**	1·03	1·30	2·02	2·53	1·73	1·64	1·15
6	4	1·13	2·90	1·97	1·40	**0·30**	**0·94**	1·40	**0·90**	2·20	**0·93**	1·97	1·37
5	5	1·17	5·17	1·11	**0·75**	**0·29**	1·25	**0·75**	**0·79**	1·25	1·00	1·39	1·19
4	6	1·20	1·82	1·23	**0·80**	**0·28**	**0·73**	**0·80**	**0·68**	1·40	1·07	2·12	1·35
3	7	1·23	1·95	**0·90**	**0·85**	**0·55**	**0·95**	1·28	**0·86**	1·03	1·13	1·79	1·01
2	8	1·27	1·63	1·47	**0·90**	1·07	1·07	**0·90**	1·40	1·13	1·20	1·66	1·12
1	9	1·30	1·58	**0·80**	**0·95**	1·03	1·73	**0·71**	1·43	**0·93**	**0·95**	1·47	**0·92**

*Ciprofloxacin used with *S. aureus* and *K. pneumoniae*. Amphotericin B used with *C. albicans*.
†*Rosmarinus officinalis* (rosemary).
‡*Melaleuca alternifolia* (tea tree).
§*Thymus vulgaris* (thyme).
¶*Mentha piperita* (peppermint).

represented scale. Amphotericin B in combination with *M. alternifolia* oil show a predominantly antagonistic profile against *C. albicans* with only two ratios (6 : 4, FIC 0·93) and (1 : 9, FIC 0·95) having a slight synergistic effect (Table 2).

The combination profiles for *T. vulgaris* (thyme) essential oil with the commercial antimicrobials are presented in Fig. 3 and Table 2. A predominantly antagonistic profile was noted against all pathogens studied. Synergy is best noted for four ratios (5 : 5, FIC 0·75; 4 : 6, FIC 0·80; 2 : 8 FIC 0·90 and 1 : 9, FIC 0·71) where *T. vulgaris* oil and ciprofloxacin are combined and tested against *K. pneumoniae* (Table 2).

In Fig. 4 and Table 2, the combination profiles for *M. piperita* (peppermint) essential oil with the commercial antimicrobial ciprofloxacin or amphotericin B is presented. Studies on *S. aureus* show a synergistic pattern for five ratios (5 : 5, FIC 0·75; 4 : 6, FIC 0·80; 3 : 7, FIC 0·85; 2 : 8 FIC 0·90 and 1 : 9 FIC 0·95). Synergy was also noted when tested against *K. pneumoniae* (ratios 6 : 4, FIC 0·90; 5 : 5, FIC 0·79; 4 : 6 FIC 0·68 and 3 : 7 FIC 0·86). However, depending on the ratio of the combination, antagonism was noted for four ratios with *S. aureus*, five ratios with *K. pneumoniae* (ratios 6 : 4, 5 : 5, 4 : 6 and 3 : 7) and all ratios when tested against *C. albicans*. Three ratios are not shown on the isobologram as they fall out of the scale presented. The best synergistic profiles noted for *K. pneumoniae* were closest to and including the 1 : 1 combination.

Discussion

The therapeutic properties of *R. officinalis* include the treatment of bronchitis, sinusitis, as an expectorant, as a mucolytic and an antiseptic. The administration by inhalation has been used by aromatherapists, traditional Chinese medicinal practitioners as well as more recently, used in the fumigation of French hospitals (Holmes 1999; Wootton 2005). *Klebsiella pneumoniae* has been identified as one of the major causes of septicaemia in paediatric wards (Boyd and Hoerl 1981). Data presented here indicates that when combining *R. officinalis* with ciprofloxacin at carefully selected concentrations, the antimicrobial efficacy against *K. pneumoniae* is enhanced.

The combination of the *M. alternifolia* essential oil with commercial antimicrobials predominantly shows an antagonistic effect. The toxicity of *M. alternifolia* oil has previously been studied and in the review by Hammer *et al.* (2006), no conclusive evidence could be found supporting toxicity, because of the complexity of the oil. Available literature does suggest, however, that dilutions applied topically have no adverse effects (Halcón and Milkus 2004). One could postulate that the combination with conventional antimicrobials may increase the therapeutic levels to unacceptable antagonistic doses, based on the assumption as stated by Guba (2001) that oil toxicity is dependent on dose and duration of exposure.

There have been a number of reports validating the antimicrobial efficacy of *T. vulgaris* (Delespaul *et al.* 2000; Valero and Salmerón 2003; van Vuuren and Viljoen 2006) and *T. vulgaris* (thyme) oil is often used in combination with other oils as a preservative however, in a recent editorial letter, a recommendation was made that thyme oil be excluded in food preservation because of possible toxicity (Eisenhut 2007). This study also suggests that thyme oil must be used with caution in combination with antimicrobials as antagonism may predominate.

The medicinal use of *M. piperita* (peppermint) essential oil includes the treatment of sinusitis by inhalation therapy (Shealy 1998) and may have potential beneficial effects when combined with ciprofloxacin to treat *K. pneumoniae* infections.

In an age where HIV infection is rife and nosocomial infections are on the increase, alternate therapies are often being sought, either in conjunction with conventional therapy or independently. Patients seeking additional therapeutic relief with popular essential oils such as those used in this study (*M. alternifolia*, *T. vulgaris, M. piperita* and *R. officinalis*), while on conventional medicine may need to be cautious, as the combination of essential oils with conventional antimicrobials does not always produce synergistic profiles as noted in previous combination studies (Shin 2003; Shin and Kang 2003; Pyun and Shin 2006; Dimitrijević *et al.* 2007; Rosato *et al.* 2007). In spite of these reports and other synergistic combinations of extracts with antibiotics (Sakagami *et al.* 2005; Yang *et al.* 2005; Inui *et al.* 2007; Okusa *et al.* 2007), it is not unusual to encounter adverse drug reactions with herbal medicines (Williamson 2001). A recent survey (Cuzzolin *et al.* 2006) highlighted the possible antagonistic interaction of phytomedicines in combination with traditional drugs, emphasising the need for more systematic interactive studies to be undertaken to identify unfavourable combinations. This *in vitro* investigation demonstrating pharmacological interactions at different ratios confirms the need to examine not only synergistic interactions but also antagonistic trends, as predominantly noted in this study. The most prevalent antagonistic interaction was noted when *M. alternifolia* oil was combined with ciprofloxacin and tested against *S. aureus*. Also, all four essential oils (*M. alternifolia*, *T. vulgaris, M. piperita* and *R. officinalis*) when combined with amphotericin B and tested against *Candida albicans* primarily showed an antagonistic profile.

It should also be noted that whether an essential oil with known antimicrobial activity will produce beneficial

or harmful effects when combined with conventional antimicrobials depends on the ratio in which the two components exist. Further research into the use of those combinations having promising synergistic profiles i.e. *M. piperita* or *R. officinalis* in combination with ciprofloxacin and tested against *K. pneumoniae* is warranted. This may in the future lead to formulations producing less side effects and having a more positive contribution in combating the ever increasing incidence of antimicrobial resistance. However, the prevalence of antagonism is noted and thus combinations of *Melaleuca alternifolia*, *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis* with ciprofloxacin or amphotericin B should be avoided.

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