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Research Article

Combined Antibacterial and Antifungal Activities of *Eucalyptus citriodora* and *Syzygium aromaticum* Essential Oils

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Background: The increasing proportion of skin infections encountered in general practice represents a substantial level of morbidity. The emergence of multi-drug resistant strains is a formidable threat to the fight against skin diseases and hence alternative forms of treatment are essential. *Syzygium aromaticum* and *Eucalyptus citriodora* oils as single entities have demonstrated potency against some of the concerned micro-organisms and any synergistic activity between the two oils could minimise development of resistance by the microorganisms to the two oils.

Objective: The aim of this study was to evaluate for synergism between *Eucalyptus citriodora* and *Syzygium aromaticum* essential oils against selected pathogenic microorganisms of the skin.

Materials and methods: *Eucalyptus citriodora* (eucalyptus oil) and *Syzygium aromaticum* (clove oil) essential oils were used in this study. *In-vitro* antimicrobial activities of *Sysygium aromaticum* and *Eucalyptus citriodora* oils, alone and in combination were tested against *Staphylococcus aureus* ATCC 25923*, Pseudomonas aeruginosa, E. coli* ATCC 25922, MRSA, *Candida albicans*, *Trichophyton mentagrophytes, Microsporum gypseum* and *Cryptococcus neoformans.*

Results: The combination of the two oils exhibited synergistic activity against *Staphylococcus aureus* (FICI: 0.240), *E. coli* (FICI: 0.54), MRSA (FICI: 0.48), and *Microsporum gypseum* (FICI: 0.36) while the combination exhibited additive activity against *Candida albicans* (FICI: 2.04).

Conclusion: The combination of clove and eucalyptus oils possesses synergistic activity against most of the test pathogens and therefore may be combined for enhanced antimicrobial activity against a wide range of skin disease-causing microorganisms.

Keywords: antifungal, antibacterial, synergism, *Eucalyptus citriodora*, *Syzygium aromaticum*, essential oils

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1. Introduction

Dermatological disorders are common in many countries (Komba and Mgonda, 2010) and are among the most frequent causes of morbidity (Accorsi et al, 2009). A variety of skin infections are seen in general practice (Sippe, 1991) and the types of organisms that cause primary skin and soft tissue infections include bacteria, viruses, fungi and parasites (Laube, 2004). Acute bacterial skin infections are common (Gabillot

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and Roujeau, 2007) and most of them are usually caused by *Staphylococcus aureus* and *Streptococcus pyogenes* (Sharma and Verma, 2001). Bacterial skin infections commonly encountered in the community include impetigo, folliculitis/furunculosis, simple abscesses and other non-necrotizing cellulitis (Bernard, 2008). Skin disorders are an important problem in children living in developing countries (Marrone et al, 2012) and those with disabilities may be particularly susceptible (Fathy et al, 2004). Methicillin-resistant *Staphylococcus aureus* has emerged as a cause of infection among otherwise healthy children and adults in the community where skin and soft tissue infections are most common, (Gorwitz, 2008). Elderly individuals have an increased susceptibility to skin infections due to age-related anatomical, physiological and environmental factors (Laube, 2004) which include thinning, decreased secretions, and reduced immune function (Weissmann, 1989). Nursing home-acquired skin and soft tissue infections are common, with an estimated prevalence of approximately 5% (Lertzman and Gaspari, 1996). New treatments for skin disease continue to evolve (Thiers, 1998) but the identification of appropriate and novel antimicrobials is continually challenged by the emergence of antimicrobial resistance among bacteria, fungi and parasites (Yang and Kerdel, 2006).

The increase in microorganisms that have developed resistance to currently available antimicrobial agents has become a major cause of concern worldwide (Finch and Hunter, 2006) and especially in Africa (Aristide et al, 2011). Bacterial infections are responsible for 90% of infections found in health care services (Lacmata et al, 2012) and the emergence of multi-drug resistant phenotypes is a major public health problem today (Fankam et al, 2011). Bacteria resistant to almost all of the available antibacterial agents have been identified (Bax et al, 2000) and serious skin and soft tissue infections caused by multidrug resistant pathogens have become more common in recent years (Raghavan and Linden, 2004). Some of the greatest global health achievements like treating tuberculosis, human immunodeficiency virus, pneumonia, diarrhoea and other infectious diseases are equally at risk (Norrby et al, 2005). Resistance of these micro-organisms to antimicrobial therapies reduces the effectiveness of the drugs leading to increased morbidity, mortality and health care expenditure (Richard and Joana, 2002).

The continuous escalation of resistant bacteria against a wide range of antibiotics has generated a considerable interest in the search for novel therapeutic compounds from medicinal plants (Njume et al, 2011). The search for drugs has accelerated in recent years and plants are amongst promising resources for finding new antibacterial agents (Aliahmadi et al, 2011) whose use as natural antimicrobial agents is gaining popularity (Fullerton et al, 2011). These natural products may give new antimicrobial agents with possibly novel mechanisms of action with the potential of addressing multiple targets with low level of resistance in addition to being cost–effective (Navan and Shukla, 2011) since therapy with synthetic antibiotics is normally associated with high cost (Aristide et al, 2011). Conventional antibiotics have certain undesirable side effects including gastric ulceration and fat redistribution (Dickson et al, 2011) coupled with many pathogens adapting evasive mechanisms against a number of compounds over time (Fowler et al, 2011). Many plant-derived essential oils are known to exhibit antimicrobial activity against a wide range of bacteria and fungi (Sean et al, 2001). They include oils from the *Eucalyptus citriodora* and clove plants.

Eucalyptus oil has a history of wide application including pharmaceutical use. It has been shown to have antibacterial effect on pathogenic micro-organisms (Salari et al, 2006). The oil has equally shown improved skin antiseptic properties when combined with chlorhexidine digluconate (Lambert et al, 2008). The clove oil from *Syzygium aromaticum* is also from the Myrtaceae family and has also been known for its analgesic, local anaesthetic, anti-inflammatory and antibacterial effects (Hemaiswarya and Doble, 2009).

The aim of this study was to evaluate for synergism between *Eucalyptus citriodora* and *Syzygium aromaticum* essential oils against selected pathogenic microorganisms of the skin.

2. Methods

2.1 Essential Oils

Eucalyptus citriodora (eucalyptus oil) and *Syzygium aromaticum* (clove oil) essential oils were used in this study. The oils were obtained as steam distillates from Bell sons and Co. Druggists Ltd (Southport, England).

2.2 Test Organisms

Four bacterial strains of *Staphylococcus aureus* ATCC 25923, methicillin resistant *Staphylococcus aureus* (clinical isolate), *Esherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* were used in the study. The fungal strains used included reference and clinical isolates of *Candida albicans* (ATCC 90028), *Cryptococcus neoformans*, and dermatophytes from clinical sources namely *Microsporum gypseum* and *Trichophyton mentagrophytes*. The fungal strains were culture collections at Mycology Laboratory, Centre for Microbiology Research, Kenya Medical Research Institute (KEMRI).

2.3 Antibacterial *In-vitro* Assay

In-vitro antimicrobial activities of clove and eucalyptus oils, alone and in combination, were performed using the disc diffusion method, while the minimum inhibition concentration (MIC) was determined by broth micro-dilution technique (Bauer, 1996; CLSI, 2003). The procedure for the *in vitro* assay was as described by CLSI (2003). Briefly, from a 24-hour culture of the test bacteria, 0.5 McFarland standard suspensions were prepared. The suspension was spread uniformly using a sterile wire loop on to a sterile Muller Hinton agar plate so as to achieve a confluent growth. Sterile 6mm paper discs impregnated with 20 µl of essential oil were then aseptically placed on the surface of the inoculated medium. The plates were then incubated at 4 °C for 1 hour to allow for diffusion of the oil into the medium and then at 37 °C for another 24 hours to enable the micro-organisms to grow and interact with the oil. The inhibition zone diameters (IZD) were measured and recorded at the end of the

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incubation time. The tests were done in triplicate and chloramphenicol (30 µg) was used as the positive control while dimethyl sulphoxide diluent (DMSO, 5 %) was used as the negative control.

2.4 Antifungal Quantitative *In-vitro* Assay

In-vitro antifungal activity of clove and *Eucalyptus* essential oils, singly and in combination was performed using disc diffusion assay and the MIC was determined using broth micro-dilution method (Bauer, 1996, CLSI 2003). The procedure for the *in vitro* assay was as described by CLSI (2003). Briefly, from a 24 hour culture of yeasts and 72 hour culture of dermatophytes, 0.5 McFarland standard suspensions were prepared and spread uniformly on Sabourauds dextrose agar. A sterile 6mm paper disc impregnated with 20 µl of the test oil was aseptically placed on the surface of the inoculated medium. Plates were incubated at 4 °C for 1 hour to allow diffusion of oil and then at 35 °C for 24 hours for yeast and at 30 °C for 72 hours for moulds. After incubation, the plates were then examined for zones of inhibition. The zone of inhibition around each disc was measured and recorded at the end of incubation time. The tests were carried out in triplicate and nystatin (23 µg) was used as positive antifungal standard while DMSO (5%) was used as negative control. All microbiological procedures were performed at the opportunistic infection laboratory at Centre for Microbiology Research. The procedures were done under level 2 containment facility and following KEMRI biosafety guidelines.

2.5 Minimum Inhibitory Concentration

To determine MIC, the oils were serially diluted in a 96 microtitre plate using DMSO (5%) as the diluents to give concentration ranges of 0.78-50 % (v/v). Sterile discs impregnated with 20 µl of the various concentrations were aseptically placed on the surface of the medium which had been inoculated with standard suspensions of the microorganisms and incubated. The lowest concentration without growth after incubation was taken as the MIC.

2.6 Combined antibacterial Activity

To assess for any synergism, the oils were mixed at various concentrations: 100:0% (v/v), 80:20% (v/v), 60:40% (v/v), 50:50% (v/v), 40:60% (v/v), 20:80% (v/v), and 0:100% (v/v) of *Eucalyptus citriodora* and *Syzygium aromaticum* oils respectively and antibacterial and antifugal activity of each concentration determined using disc diffusion method. Chequerboard assay was used to assess for synergistic activity of *Eucalyptus citriodora* in combination with *Syzygium aromaticum*.

2.7 Minimum Bactericidal and Fungicidal Activity of *Eucalyptus citriodora* and *Syzygium aromaticum* oils

To determine the minimum bactericidal (MBC) or minimum fungicidal concentrations (MFC), the oils were serially diluted in a 96 microtitre plate after which 100 µl of bacterial or fungal standard suspension was added into each serial dilution. To each microwell, 100 µl of Muller Hinton agar (for MBC) or Sabouraud's dextrose agar (MFC) was added. The mixture was incubated at respective conditions. At the end of the incubation period, the mixture from each microwell was picked using a sterile wire loop and applied on the surface of antibiotic-free media. Muller Hinton and Sabouraud's dextrose agar were used for bacteria and fungi respectively. The plates were then incubated at the recommended temperature and time. At the end of the incubation period the lowest concentration that prevented growth was taken as the MBC or MFC.

2.8 Statistical Analysis

The inhibition zone diameter means and the standard deviations of *Syzygium aromaticum* and *Eucalyptus citriodora* oils when acting alone and in combination against the tested micro-organisms were computed and used to compare their antimicrobial activities. Student's t -test test was used to ascertain if the p values from inhibition zones were statistically significant at 95% confidence level ($p<0.05$). The minimum inhibitory concentrations (MIC) were evaluated and used to compare the effectiveness of the oils against the test pathogens. Chequerboard assay was performed and Fractional inhibitory concentration index (FICI) computed and used to determine exhibition of any synergistic activity between the two oils.

3. Results

In-vitro Inhibitory Activity of *Syzygium aromaticum* and *Eucalyptus citriodora* Oils against the Test Bacteria.

Both *Syzygium aromatium* and *Eucalyptus citriodora* essential oils were found to be active against nearly all the test bacteria (**Table 1**). Based on the inhibition zone diameter (IZD) values, the microorganisms were placed into four categories: microorganisms having IZD ≤ 8 mm, considered resistant; microorganisms with IZD between 9-14 mm, considered sensitive; microorganisms with IZD between 15-19 mm, considered very sensitive; while microorganisms with IZD ≥ 20 mm were considered extremely sensitive to the oil (Babu et al, 2011).

From the results MRSA and *E. coli* were found to be extremely sensitive to *Eucalyptus citriodora* oil. *Staphylococcus aureus* was highly sensitive to *Eucalyptus citriodora* oil as well as *Syzygium aromaticum* oil while *Pseudomonas aeruginosa* was resistant to the two oils. *E. coli* and MRSA were found to be very sensitive to *Syzygium aromatic* oil. MRSA, *E. coli* and *Staphylococcus aureus* were extremely sensitive to chloramphenicol while *Pseudomonas euruginosa* was very sensitive to this reference drug.

There was a significant difference ($p\leq0.05$) in activity between the oils against MRSA with *Eucalyptus citriodora* oil showing higher activity ($p=0.013$) than *Syzygium aromaticum* oil against the bacteria. However, there was no difference ($p\leq0.05$) in activity between the oils against *Staphylococcus aureus* ($p=0.57$) and *E. coli* ($p=0.281$). Chloramphenicol showed higher activity against all the test bacteria than the two oils.

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Table 1: Mean diameter of zones of inhibition (mm) of *Syzygium aromaticum* and *Eucalyptus citriodora* oils at neat concentrations against the test bacteria and fungi

Microorganism	*Syzygium aromaticum*	*Eucalyptus citriodora*	Chloramphenicol	Nystatin
Bacteria				
Staph. aureus	*19±1.9	*18±2.2	25	
E. coli	*18±2.0	*20±1.9	25	
MRSA	**16±2.1	**23±1.9	28	
P. aeruginosa	*8±1.9	*8±2.0	19	
Fungi				
M. gypseum	**11±2.4	**20±2.0		19
T. mentagrophytes	*18±1.8	*14±2.2		27
C. albicans	*15±2.0	*18±2.0		22
C. neoformans	**15±2.0	**10±0.4		21

Chloramphenicol was used as positive control and DMSO as the negative control.
Each value is the average of three independent replicates.
**Antimicrobial activity not significantly different at p≤0.05.*
***Antimicrobial activity significantly different at p≤0.05.*

Table 6: The minimum inhibitory concentration of *Eucalyptus citriodora* when combined with *Syzygium aromaticum* at various concentrations against the test microorganisms (%v/v)

Microorganism	100:0	80:20	50:50	60:40	40:60	20:80	0:100	MIC (%v/v)
Sa	24	19	14	11	10	9	0	**0.25**
Ec	21	16	11	10	9	0	0	**0.67**
MRSA	22	17	12	10	0	0	0	**1.5**
Mg	23	18	13	9	0	0	0	**1.5**
Ca	19	14	10	9	0	0	0	**1.5**

Cn-Cryptococcus neoformans; MRSA-*Methicillin-resistant Staphylococcus aureus; Sa-Staphylococcus aureus; Ec- E. coli; Tm Trichophyton mentagrophytes*; *Mg- Microsporum gypseum*

NB: Test not determined for *Pseudomonas aeruginosa, Candida albicans* and *Trichophyton mentagrophytes.*

In-vitro Inhibitory Activity of *Syzygium aromaticum* and *Eucalyptus citriodora* Oils against the Test Fungi

Syzygium aromaticum and *Eucalyptus citriodora* oils exhibited significant activity against the test fungi (**Table 1**).

Candida albicans was found to be very sensitive to *Syzygium aromaticum* as well as *Eucalyptus citriodora* oil. *Trichophyton mentagrophytes* and *Cryptococcus neoformans* were found to be very sensitive to *Syzygium aromaticum* oil. *Microsporum gypseum* was extremely sensitive to the *Eucalyptus citriodora* oil while *Trichophyton mentagrophytes* and *Cryptococcus neoformans* were sensitive to the oil.

Microsporum gypseum was very sensitive while *Trichophyton mentagrophytes*, *Candida albicans* and *Cryptococcus neoformans* were extremely sensitive to nystatin.

There was a significant difference (p≤0.05) between the activity of the oils against *Microsporum gypseum* and *Cryptococcus neoformans* with *Eucalyptus citriodora* oil being more active (p=0.008) against *Microsporum gypseum* while *Syzygium aromaticum* demonstrated better potency (p=0.013) against *Cryptococcus neoformans*. Nystatin showed higher activity than the oils against *Trichophyton mentagrophytes*, *Cryptococcus neoformans* and *Candida albicans*.

Minimum inhibitory concentrations of *Syzygium aromaticum* and *Eucalyptus citriodora* oil against the test microbes.

The *Syzygium aromaticum* oil exhibited the lowest MIC against *Candida albicans* (0.78 % v/v) and the highest MIC against *Trychophyton mentagrophites* (12.5% v/v) (**Table 2** – Supporting information). On the other hand, the *Eucalyptus citriodora* oil exhibited the lowest MIC (3.125% v/v) against *Staphylococcus aureus* while

acting at a higher MIC (12.5% v/v) against *Microsporum gepseum* and *Candida albicans* (**Table 3** – Supporting information). *Staphylococcus aureus*, *E.coli*, *Microsporum gypseum* and *Candida albicans* exhibited sensitivity to *Syzygium aromaticum* oil at lower concentrations compared to *Eucalyptus citriodora* oil.

Minimum Bactericidal and Minimum Fungicidal Activity of *Syzygium aromaticum* and *Eucalyptus citriodora* oil

The minimum bactericidal and minimum fungicidal concentrations of the oils were determined at various concentrations**.** The concentration where no growth occurred the oil was deemed to be bactericidal or fungicidal while it was considered to be bacteristatic or fungistatic where growth occurred after reculturing. From the findings the oil showed bactericidal and fungicidal effects at varied concentrations against the test pathogens (**Tables 4** and **5** – Supporting information). The *Syzygium aromaticum* oil exhibited the lowest bactericidal concentrations of 1.56 % (v/v) against *Staphylococcus aureus* and MRSA and the lowest fungicidal concentration of 6.25%(v/v) against *Microsporum gypseum. Eucalyptus citriodora* oil exhibited the lowest bactericidal concentration of 6.25% (v/v) against *Staphylococcus aureus* and MRSA and lowest fungicidal concentration against *Microsporum gypseum* (6.25% v/v). *Eucalyptus citriodora* was more active against *Cryptococcus neoformans* than *Syzygium aromaticum*; however *Syzygium aromaticum* was more active against *Staphylococcus aureus*, MRSA, *Trychophyton mentagrophytes* and *Candida albicans*.

Combined Antimicrobial Activity of *Eucalyptus citriodora* with *Syzygium aromaticum* oil

The antimicrobial activity of *Eucalyptus citriodora* oil in combination with *Syzygium aromaticum* oil (at ratios of 100:0, 80:20, 50:50, 60:40, 20:80 and 0:100) was determined against the test microorganisms. From the findings *Staphylococcus aureus* was found to be most sensitive to the combination with the lowest MIC of 0.25 %(v/v) followed by *E. coil* (MIC: 0.67% v/v). MRSA, *Mycrosporum gypseum* and *Candida albicans* each exhibited an MIC of 1.5% (v/v).The combination of the two oils exhibited activity against *Staphylococcus aureus*, *E. coil*, MRSA and *Mycrosporum gypseum* at lower concentrations than the individual oils (**Table 6**).

Table 7: Chequer board Determination of the Combined Antimicrobial Activity of *Eucalyptus citriodora* and *Syzygium aromaticum oils*.

Mo/Cn	MIC of EC (%v/v) alone/in combination		FIC of EC	MIC of SA *oil* (%v/v) alone/ combination		FIC of SA	FICI	Result
Sa								
EC+SA	3.125	0.25	0.08	1.56	0.25	0.16	0.24	Synergistic
Ec								
EC+SA	6.25	0.67	0.11	1.56	0.67	0.43	0.54	Synergistic
MRSA								
EC+SA	6.25	1.5	0.24	6.25	1.5	0.24	0.48	Synergistic
Pa								
EC+SA	Nd		Nd	Nd	Nd	Nd	Nd	Nd
Mg								
EC+SA	12.5	1.5	0.12	6.25	1.5	0.24	0.36	Synergistic
Ca								
EC+SA	12.5	1.5	0.12	0.78	1.5	1.92	2.04	Additive
Tm								
EC+SA	12.5	Nd	Nd	12.5	Nd	Nd	Nd	Nd
Cn								
EC+SA	3.125	Nd	Nd	3.125	Nd	Nd	Nd	Nd

Nd- Not determined.; Mo- Microorganisms; EC- Eucalyptus citriodora;
FIC- Fractional inhibitory concentration; FICI- Fractional inhibitory concentration index
Cn- Combination of oils ; SA- Syzygium aromaticum

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Synergistic Antimicrobial Activity between *Eucalyptus citriodora* and *Syzygium aromaticum*

From the various concentrations (Table 7) the Chequerboard assay was used to assess for synergism between the two oils. For each microorganism, the *Eucalyptus citriodora*: *Syzygium aromaticum* oil combination was deemed synergistic, additive or antagonistic by calculating the Fractional Inhibitory Concentration (FIC) for each agent and then the FIC index (FICI) using the following formulae:

FIC= The MIC of antimicrobial in combination/ MIC of antimicrobial alone.

FICI= FIC of *Eucalyptus citriodora* + FIC of *Syzygium aromaticum oil*.

FICI≤ 0.5, synergistic; FICI> 0.5 or ≤ 4.0, additive; FICI> 4.0, antagonistic (Henry *et al*, 2009).

From the results synergistic activity was demonstrated with *Staphylococcus aureus* (FICI: 0.240, *E. coli* (FICI: 0.54) MRSA (FICI: 0.48), and *Microsporum gypseum* (FICI: 0.36) while the combination exhibited additive activity against *Candida albicans* (FICI: 2.04).

4. Discussion

***In-vitro* Inhibitory Activity of *Syzygium aromaticum* and *Eucalyptus citriodora* Essential Oils Against the Test Bacteria and Fungi**

The antimicrobial activity of plant oils and extracts has been recognized for many years (Hammer *et al*, 1999) and essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Seenivasan *et al*, 2006). However no study appears to have been done on synergistic antibacterial and antifungal activity of *Syzygium aromaticum* and *Eucalyptus citriodora* oils. In this study antibacterial and antifungal activity of the oils alone and in combination were tested. From the results, the two essential oils exhibited significant antimicrobial activity against nearly all the test bacteria. This concurs with earlier observations (Hammer et al, 1999; Sean et al, 2001) that many plant- derived oils exhibited antimicrobial activity against a wide range of bacteria and fungi. *Eucalyptus citriodora* oil contains terpenes which are known to be active against a wide variety of microorganisms, including gram-positive and gram-negative bacteria and fungi (Trombetta, 2005) and this may account for the broad spectrum of activity of *Eucalyptus citriodora* oil as documented in this study.

Pseudomonas aeruginosa was, however, shown to be resistant to the two oils and this observation concurs with findings of Gupta et al (2009) and Javad et al (2010). Moshi et al (2007) had similarly observed increasing resistance by *Pseudomonas aeruginosa* to conventional antibiotics such as norfloxacin, ciprofloxacin and amoxicillin-clavulanic acid. According to Abdel-Hameed (2003), this phenomenon of antimicrobial resistance continues to increase and many diseases are becoming increasingly difficult to treat as medicines become less effective resulting in a steady depletion of otherwise potent drugs that are currently available. The resistance of *Pseudomonas aeruginosa* appears to be the result of an external membrane particularly impermeable to essential oil molecules and the presence of efflux mechanisms and porine-dependent inhibition that protect the bacteria against the action of the oils (Mayaudi *et al* 2008). More research work that could involve chemical structural modification could therefore be necessary in order to evade the targeting of the drug for elimination by the microorganism. The microbe was however found to be very sensitive to chloramphenicol.

Trivedi and Hotchandani (2004) however found *Eucalyptus* oil to be active against *Pseudomonas aeruginosa* while Dorman and Deans (2000) and Bari et al (2004) found *Syzygium aromaticum* oil to be active against the microorganism. The oils were found to be effective against MRSA which is supported by earlier observations made by Tohidpour et al (2010) and Sporer et al (2011) and in addition, MRSA was found to be very sensitive to *Syzygium aromaticum* oil thereby concurring with Babu et al (2011). The microbe was also found to be extremely sensitive to *Eucalyptus citriodora* oil and as Trivedi *et al* (2004) noted, topical application of *Eucalyptus* oil proved effective against MRSA.

There was a significant difference (p≤ 0.05) in the activity between the oils against MRSA with *Eucalyptus citriodora* exhibiting better activity. This suggests that given a choice between the two oils in the management of suspected MRSA infection, *Eucalyptus citriodora* would do better. *Staphylococcus aureus* was equally very sensitive to *Eucalyptus oil* and extremely sensitive to chloramphenicol. However, Javad and Batooli (2010), Babu et al (2011) and Trivedi and Hotchandani (2004) found *Staphylococcus aureus* to be extremely sensitive to both oils. Kamal and Randhika (2010) however found *Syzygium aromaticum* oil to be inactive against *Staphylococcus aureus*.

Both oils were also found to be active against *E. coli* and this is in agreement with observations made by Trivedi and Hotchandani (2004), Javad and Batooli (2010), Gupta et al (2009) , Ayoola et al (2008) and Sudsadu et al (2010). *E. coli* was found to be extremely sensitive to *Eucalyptus citriodora* oil while Javad and Batooli (2010) observed the microbe as being very sensitive to the oil. The microbe was also very sensitive to *Syzygium aromaticum* oil which is in agreement with Burt and Reinders (2003) and Gupta et al (2009).

Syzygium aromaticum oil and *Eucalyptus citriodora* oils were equally found in this study to be effective against the test fungi. Previous study (Eugénia, 2009) indicated that *Syzygium aromaticum* (clove) and eugenol have considerable antifungal activity against clinically relevant fungi, including fluconazole-resistant strains. The oils were found to be active against *Microsporun gypseum*. The microbe in this study was found to be sensitive to *Syzygium aromaticum* oil and extremely sensitive to *Eucalyptus* oil. The sensitivity observed concurs with that of Park et al (2007) and Pinto et al (2009). M*icrosporun gypseum* was equally very sensitive to nystatin. *Trichophyton mentagrophytes* was also found to be very sensitive to *Syzygium aromaticum* oil in addition to being sensitive to *Eucalyptus ciriodora* oil thereby concurring with observations of Park et al (2007) and Lugman et al (2008). *Trichophyton*

mentagrophytes was also extremely sensitive to nystatin. *Candida albicans* was found to be very sensitive to *Syzygium aromaticum* oil, *Eucalyptus citriodora* and extremely sensitive to nystatin. The sensitivity of the micro-organism to both oils concurs with earlier observations by Ayoola et al (2008), Park et al (2007), Pinto et al (2009), Briozzo et al (1998), Javad and Batooli (2010) Lugman et al (2008) and Rasooli et al (2009). However, Kamal and Radhika (2010) found *Syzygium aromaticum* to be inactive against *Candida albicans*. Both oils exhibited activity against *Microsporum gypseum* which is in agreement with observation by Lugman et al (2008). The micro-organism was found to be extremely sensitive to nystatin. *Cryptococcus neoformans* was sensitive to *Syzygium aromaticum* oil and *Eucalyptus citriodora* oil and this was equally demonstrated by Lugman et al (2008). There was, however, no significant difference ($p \leq 0.05$) in activity between the two oils against S*taphylococcus aureus* and *E. coli*. The pathogen was equally extremely sensitive to chloramphenicol.

Eucalyptus citriodora oils was also found to be effective against S*taphylococcus aureus* and this observation concurs with those of Ayoola et al (2008), Rasooli et al (2009), Dorman and Deans (2000) and Bari et al (2008). The micro-organism was also found to be very sensitive to *Syzygium aromaticum* oil which is in concurrence with observation by Gupta et al (2009).

The fact that the two oils exhibited significant activity against the test microorganisms may suggest the usefulness of the oils in combating varied skin diseases. Moreover, these oils are cheap, readily available, easy to prepare and less toxic compared with conventional agents. In addition, there has been an increase in the number of microorganisms that have developed resistance to conventional drugs (Finch and Hunter, 2006).

Minimum Inhibitory Concentrations of *Syzygium aromaticum* and *Eucalyptus citriodora* Essential Oils Against the Test Bacteria and Fungi.

Syzygium aromaticum was most effective against *Staphylococcus aureus* and *E. coli* than MRSA. The activity against *Staphylococcus aureus* by the oil was lower than that observed by Sudsadu et al (2010). The oil was also most effective against *Candida albicans* compared with other fungi. *Eucalyptus citriodora* was also found to be most effective against *Staphylococcus aureus* and least effective against *Microsporum gypseum* and *Candida albicans*. *Syzygium aromaticum* was however effective at lower concentrations against *Staphylococcus aureus*, *E. coli*. *Microsporum gypseum* and *Candida albicans* meaning that as monotherapies *Syzygium aromaticum* will be better in managing infections caused by any of these pathogens. From these findings the two oils exhibit effectiveness at fairly low concentrations against the test microorganisms.

Minimum Bactericidal and Minimum Fungicidal Activity of *Eucalyptus citriodora* and *Syzygium aromaticum* oils

The *Eucalyptus citriodora* oil was more active against MRSA and *Staphylococcus aureus* than *E. coli*. However the sensitivity of MRSA to the oil at fairly low concentrations suggests that it can be a cheap alternative in managing intractable infections especially of the skin associated with the pathogen. The oil exhibited activity against *Microsporum gypseum, Cryptococcus neoformans, Candida albicans* and *Trychophyton mentagrophytes*. Arising from these observations *Eucalyptus citriodora* oil may be useful in managing fungal skin diseases such as *Tinea cruris*, *Tinea capitis, Tinea versicolar* or *Tinea corporis* and other opportunistic fungal infections. *Syzygium aromaticum* oil exhibited good activity against *Staphylococcus aureus* and MRSA. The oil also exhibited good fungicidal activity against *Microsporum gypseum*, suggesting that fungal infections particularly caused by *Microsporum gypseum* respond well to the oil.

Combined Antimicrobial and Synergistic Activity of *Eucalyptus citriodora* with *Syzygium aromaticum* oil

Two drugs applied to a system can either give the same response as the sum of the two drugs individually (additive), a greater response (synergistic) or a smaller response (if one drug blocks the effects of the other) (Vijaya *et al*, 2007).

In this study, the two oils were combined at different concentrations and their antibacterial and antifungal activity tested. The combination was however effective at lower concentrations against *E. coli*, MRSA, *Microsporum gypseum* and *Candida albicans* when compared with the individual oils indicating that better results would be achieved from the combination than if the oils were used individually.

The Chequerboard assay was used to assess for synergism between the two oils. From the results synergistic activity was demonstrated with *Staphylococcus aureus*, *E. Coli,* MRSA, and *Microsporum gypseum*. These observations suggest that the oils augment each other in their activity against these pathogens and hence better therapeutic response would be achieved with the combination as opposed to using the individual oils to treat conditions associated with the susceptible microorganisms. The combination however exhibited additive activity against *Candida albicans* suggesting that a combination of the two oils may not be of any significance in diseases associated with the yeast.

5. Conclusion

This study confirms that both *Eucalyptus citriodora* and *Syzygium aromaticum* oils are effective against the test microorganisms and hence may present an alternative source of natural antimicrobial agents for use in the management of skin diseases. The combination of the two oils is also effective but at lower concentrations than the individual oils and in addition exhibit synergistic activity against *Staphylococcus aureus*, *E. coli*, MRSA and *Microsporum gypseum* and therefore may be combined for enhanced antimicrobial activity against a wide range of skin disease-causing microorganisms.

However stability profile studies could be undertaken to ascertain the possibility of formulating a product from the essential oils that can be clinically applied.

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Conflict of Interest declaration

The authors declare no conflict of interest

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