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Full Length Research Paper

Antimicrobial activities and time kill profiles of five essential oils from Southern Africa against selected bacterial and fungal organisms

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Essential oils are complex mixtures of volatile secondary metabolites from plants. In this study, essential oils from five plants traditionally used to treat infectious diseases were tested for antimicrobial activity against seven Gram-positive bacteria, eight Gram-negative bacteria and six yeast species (*Candida* species and *Cryptococcus neoformans*) using the agar diffusion method. The minimum inhibitory concentrations (MIC) of the oils were determined by the microdilution technique. The killing kinetics of the oils was further evaluated against specific bacterial and fungal organisms. Both antifungal and antibacterial activities were observed from the essential oil *of Conyza scabrida*, *Eriocephallus punculatus* and *Artemisia afra* with the MIC values ranging from 0.95 to 7.5 mg/ml against the bacterial isolates and 0.24 to 7.50 mg/ml against the fungal isolates. The oils of *Adansonia digitata* and *A. afra* were fungicidal to all the yeast isolates tested with minimum fungicidal concentration (MFC) values ranging from 0.12 to 7.50 mg/ml, while the essential oil from *C. scabrida* was fungicidal to 4 of the 6 yeast isolates tested with the smallest MFC of 0.48 mg/ml against *Candida tropicalis*. Essential oils from *A. afra* were able to kill 90% of the *Pseudomonas aeruginosa* cells within 3 h. This study revealed the antimicrobial activity of *C. scabrida* and *Helichrysum foetidum*. The results of this study indicate that essential oils are promising sources of natural products with potential antimicrobial activity. These results will guide the selection of some plant species for further pharmacological and phytochemical analysis. These results also support the use of essential oils to treat microbial infections and could be used as pharmaceuticals as well as preservatives in the food industry.

Key words: Medicinal plants, essential oils, antibacterial activity, antifungal activity, time-kill activity.

INTRODUCTION

Essential oils are complex mixtures of volatile secondary metabolites from plants. They have been used for years for beauty, but also to treat and control various infectious diseases (Moghaddasi, 2010; Gundidza et al., 2009). They are found in many plants and almost any organ of the plant may be source of the oil. Examples are flowers

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(Rose), leaves (mint), fruits (Lemon), bark (Cinnamon), wood (Cedar), root (Ginger), or seeds (Cardamon) and many exudations as well (Okigbo et al., 2009). Some of these oils also possess a pleasant taste and strong odour of aromatics which make them suitable for use in the cosmetic industry (Kalemba and Kinicka, 2003). Essential oils are known to have a variety of pharmacological effects, including anti-inflammatory, anti-viral and anti-microbial activities (Naimi et al., 2003). The oil may also have some antiseptic or bactericidal value. Secondary metabolites of essential oil are responsible for both fragrances and biological effect of aromatic medicinal plant (Angioni et al., 2003).

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. With the advent of the HIV and AIDS epidemic, there has been emergence and re-emergence of several microorganisms responsible for opportunistic infections in these patients coupled with an increase in drug resistance (Ahmad and Beg, 2001). Therefore, there is need to develop new substances that can be used for the control of these infections. Down the ages, essential oils have evoked interest as sources of natural products used to fight against various infectious diseases. Essential oils are a rich source of biologically active compounds and several essential oils have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Gundidza et al., 2009; Meftahizade et al., 2011). The World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare, because this is cheaper and is accessible to most individuals within the community (WHO, 2006).

Due to the presence and increase of numerous drug resistant strains, an urgent need exist to develop novel antimicrobial agents (Naimi et al., 2003; Njume et al., 2011; Syed et al., 2011). Several studies have shown that essential oils have antimicrobial activities including African essential oils. However, less than 10% of the biodiversity in Africa is said to have been evaluated. Essential oil derives their antibacterial effect from their unique chemical makeup. Each single, pure essential oil consists of several chemical compounds, sometimes hundreds of distinct natural chemicals (Chalannavar et al., 2011). Many of these compounds have antibacterial activity, and show synergistic effects all together as blends of the chemicals as found naturally in oil, and the total can be more potent than any individual chemical alone. Many studies have shown that caryacrol, the primary molecule found in oregano oil has exceptionally strong antimicrobial activity (Govaris et al., 2009). Further studies noted the combination of caryacrol and thymol to be more potent than either one alone (Byoul et al., 2007). Several studies in the African Continent have determined the antibacterial activities of essential oils (Oladipupo and Adebola, 2009; Samie et al., 2009; Gundidza et al., 2009). However, further studies are needed in order to provide more data on the rich biodiversity of the mother continent. This study determines the biological activities of different essential oils from the Southern African region against bacterial and fungal organisms pathogenic to man and animals.

MATERIALS AND METHODS

Preparation of essential oil

Medicinal plants commonly used by the Southern African region for the treatment of different infectious diseases were selected to be used in this study from the literature. A total of 9 plants were selected. Table 1 shows the name of the plants used in the present study. The plants were all collected in Zimbabwe around the city of Harare. Essential oils were prepared by hydro-distillation method. Briefly, 200 g of fresh plant parts were submitted to hydro-distillation with a Clevenger-type apparatus and according to the European Pharmacopoeia, and were extracted with 2 L of water for 3 h. Nine essential oils were prepared from the leaves of the plants. They were collected and dried under anhydrous sodium sulphate and were stored at 4°C until it was used.

Preparation of microorganisms

In order to evaluate the antimicrobial activity of essential oil, 26 organisms were used including Gram positive and Gram negative bacteria (14 in total) and yeast isolates (*Candida* species and *Cryptococcus neoformans*). The bacterial organisms were both clinical isolates and standard isolates from ATCC (Table 2). Prior to the test, a 0.5 McFarland standard to the bacterial cell were prepared in Muller-Hinton broth according to NCLS standards. The yeast organisms were all clinical isolates obtained from HIV and AIDS patients.

Antibacterial activity

Determination of minimum inhibitory concentration (MIC)

Microdilution method was used to determine the MIC as previously described (Eloff, 1998) with the following modifications: a final concentration of 0.05% (v/v) Tween-20 (Sigma) was incorporated into the medium to enhance oil solubility. Briefly, 185 µl of brain heart infusion broth (containing 0.5% Tween-20) was placed in the first well of each column of the microtitre plates and 100 µl in the rest of the wells. Fifteen microlitres of essential oil was placed in the first well and was mixed. Thereafter, 100 µl of the mixture was transfer to the next well and the same procedure was repeated until the last well to achieve a serial two fold dilution in the wells. 100 µl of the microorganism's culture was added to the broth. The plates were then incubated at 37°C until the next day. At the end of the incubation period, 50 µl of iodo-nitro tetrazolium (INT) was added to each well. MIC was determined as the smallest concentration of the extracts that inhibited the growth of the organisms (Motsei et al., 2003).

Determination of minimum bactericidal concentration (MBC)

The microtitre plates previously used to determine the MIC of the

Table 1. Ethnobotanical information on plants used for the preparation of essential oils.

Scientific name	Common name	Source	Applications
A. digitata	Boabab oil	Seeds	Traditionally used for prophylaxis and cure of dry and thin skin
A. afra	Wormwood	Leaves	For the treatment of cough, croup, whooping cough, influenza, fever, diabetes, gastro-intestinal disorders and intestinal worms
C. scabrida	Gozo-plant	leaves	Help in the production of pharmaceutical products such as antibiotics
E. punculatus	Cape Chamomile	Stem and leaves	The leaves of *E. punculatus* are used in baths for its relaxing and invigorating scent. It is also used in pillows, the scent encourages pleasant dreams. The fumes of the burning fresh plant are used to disinfect the house and clear evil spirits after a death has occurred.
H. foetidum	Strawflower and everlasting	leaves	*Helichrysum* spp. are used as food plants by the larvae of some *Lepidoptera* species

Table 2. List of the bacterial and fungal organisms used in the study.

Bacteria	Origin	Type of organism
Acinetobacter calcoaceticus	Clinical isolate	Gram-negative
B. cereus	Clinical isolate	Gram-positive
E. coli	ATCC 8739	Gram-negative
E. coli	Clinical isolate	Gram-negative
Klebsiella pneumoniae	Clinical isolate	Gram-negative
M. kristinae	Clinical isolate	Gram-negative
P. vulgaris	ATCC 6830	Gram-negative
P. aeruginosa	ATCC 7700	Gram-negative
Salmonella species	Clinical isolate	Gram-negative
S. typhi	Clinical isolate	Gram-negative
Serratia marsecens	ATCC 9986	Gram-negative
S. aureus	Clinical isolate	Gram-positive
S. epidermidis	Clinical isolate	Gram-positive
Streptococcus faecalis	ATCC 29212	Gram-positive
C. albicans	Clinical isolate	Yeast
C. glabrata	Clinical isolate	Yeast
C. kruzei	Clinical isolate	Yeast
C. parapsilosis	Clinical isolate	Yeast
C. tropicalis	Clinical isolate	Yeast
C. neoformans	Clinical isolate	Yeast

essential oils were used to determine the MBC. The wells in plates that showed no visible growth were inoculated onto 250 mm Mueller-Hinton agar plates and were incubated overnight at 37°C, and the plates were observed for growth the next day. The cultures from the wells with the smallest concentration that did not show any growth on the agar plates were recorded as MBC (Yaya et al., 2008).

Killing curve determination

Sterile 96 well microtitre plates were used with fresh brain heart infusion broth for the determination of the killing curves. Briefly, 180 µl of sterile freshly prepared brain heart infusion broth was added to the wells. Twenty microlitres of essential oil was added to the media (containing 0.5% Tween-20) inside the wells. 100 µl of the organism in brain heart infusion was added to each well such that each well finally contains the volume of 300 µl. Each test was run in two different wells. At specific time period, 10 µl of the mixture from each well was added to a new plate and the volume was adjusted to 200 µl with sterile distilled water and the optical density (OD) was read using the enzyme-linked immunosorbent assay (ELISA) reader at 590 nm. The experiment was repeated every 3 h for 2 days.

Antifungal activities

Hole-plate diffusion method

The antimicrobial activity of the essential oils was assayed by a modification of the agar diffusion method (Kirby‑Bauer). The experiments were conducted on Sabouraud Dextrose agar (SDA) plates supplemented with 0.5% Tween-20. Briefly, SDA plates were inoculated with 1000 µl of a 1 McFarland standard of the organisms grown in brain heart infusion broth (Apak and Olila, 2006). Afterward, 6 wells of approximately 5 mm in diameters and 2.5 mm deep were made on the surface of the solid medium using the tip of a sterile plastic pipette. Each well was then filled with 20 µl of the test oil or controls. Sterile dimethylsulfoxide (DMSO) was used as negative control and Nystatin was used as positive control. The plates were then incubated at 30°C for 2 to 3 days. After 3 days, the radial zone of inhibition was measured by using a ruler and the diameters of inhibition zone were determined in millimeters. Essential oils with zone of inhibition greater or equal to 6 mm diameter were regarded as active.

Determination of minimum fungicidal concentration (MFC)

The microtitre plates previously used to determine the MIC of the oils were used to determine the MFC (Yaya et al., 2008). The wells in the plates showing no visible growth were inoculated onto a potato dextrose agar plate as described earlier. The Petri dishes were marked according to the number of wells and essential oil appearing on the microtitre plates. The plates were incubated at 30°C for 2 days. The smallest concentration that did not show any growth on the agar plates was regarded as the MFC.

Killing curve determination

Sterile microtitre plates, of 96 wells were used with fresh brain heart infusion broth for the determination of the killing curve (Samie et al., 2009). Briefly, 200 µl of sterile fresh brain heart infusion broth was added to the wells together with 100 µl of the fungal culture. About 20 µl of extracts was added to the wells and the plates were incubated at 30°C. Ten microlitres of the mixture from the first plate was transfer to a new microtitre plate with 200 µl of sterile distilled water and the OD was read using ELISA reader every day for 6 days. All the experiments were repeated twice.

Statistical analysis

All the tests were conducted in duplicates. The data were analyzed using the Statistical Package for Social Sciences (SPSS) program. Chi square was used and p values were determined. The difference between two variables was considered significant when the p value was less than 0.05.

RESULTS

Antibacterial activity of the essential oils

Of all the essential oils tested, the oil of *Conyza scabrida* were the most active with MIC varying from 0.95 to 7.50 mg/ml against 11 of the bacterial strains used in this study. *C. scabrida* gave a MIC less than 1 mg/ml against 6 different bacterial organisms, while *Artemisia afra* gave an MIC of 3.75 mg/ml against 4 bacterial strains. These oils were followed by that of *Adansonia digitata* which was active against 9 different bacterial strains with MIC values ranging from3.75 to 7.5 mg/ml. Table 3 shows the MIC of the essential oils against the bacterial organisms. Of all the organisms tested, *Escherichia coli* strains, both the standard ATCC strain and the clinical strain were the most resistant to the essential oils while *Micrococcus kristinae* (Clinical isolate). Both strains of *Salmonella typhi* (clinical isolates) had similar resistance profiles to the essential oils except for *A. afra* which was active against one strain and not against the other.

Bactericidal activity of the essential oils

Table 4 shows the minimum bactericidal concentrations of the essential oils against the bacterial organisms tested. Of all the nine essential oils tested for the bactericidal activity, essential oil of *C. scabrida* was the most active with bactericidal activity against 8 different bacterial strains with MBC values ranging from 0.95 to 7.50 mg/ml. The essential oil of this plant showed an MBC less than 1 mg/ml against 4 different bacterial organisms including *Proteus vulgaris, Staphylococcus epidermidis* and the 2 *S. typhi* strains (both clinical isolates). Essential oils of both *Helichrysum foetidum* and *A. afra* were active against three bacterial strains with MBC values varying from 3.75 to 7.50 mg/ml. However, the essential oils from *Eriocephallus punculatus* were not bactericidal against any of the bacterial strains tested in this study. *S. epidermidis* was the most susceptible organism to the essential oils as it showed MBC values less than 1 mg/ml to 2 different essential oils including *C. scabrida*.

Killing kinetics of the bacterial organisms by the essential oils

Four different essential oils were tested for killing activity against seven bacterial strains. Essential oils that were tested for killing curve were those that gave MBC values less than 7.5 mg/ml against the bacterial organisms. The essential oil tested included those of *A. afra, A. digitata, C. scabrida* and *H. foetidum*. The bacterial strains tested included *Bacillus cereus, P. vulgaris, Staphylococcus aureus, S. epidermidis,* and *Pseudomonas aeruginosa* since these are the organisms that were susceptible to the essential oils. Figures 1 and 2 show the killing curves of the bacterial organism by the essential oils.

Three essential oils, *C. scabrida, H. foetidum,* and *A. afra,* were tested against *P. aeruginosa.* The essential oils from all the three plants were able to significantly reduce the number of cells just after 3 h, while in the mean time, the negative control did not stop the growth of the organisms instead, the organisms continued to grow. *A. afra* essential oils were able to kill 60% of the *P.*

Table 3. Minimum inhibitory activity of the essential oils against the bacterial organisms.

Microorganism	*C. scabrida*	*H. foetidum*	*A. digitata*	*E. punculatus*	*A. afra*
Acinetobacter calcoaceticus	3.75	>7.50	7.50	7.50	>7.50
B. cereus (clinical isolate)	0.95	3.75	3.75	>7.50	3.75
E. coli (ATCC 8739)	>7.50	>7.50	>7.50	>7.50	>7.50
E. coli (clinical isolate)	>7.50	7.50	>7.50	>7.50	>7.50
Klebsiella pneumoniae (clinical isolate)	7.50	>7.50	7.50	7.50	>7.50
M. kristinae (clinical isolate)	3.75	7.50	7.50	7.50	>7.50
P. aeruginosa (ATCC 7700)	0.95	3.75	3.75	7.50	3.75
P. vulgaris	0.95	3.75	>7.5	7.50	>7.50
Streptococcus faecalis (ATCC 29212)	7.50	>7.5	3.75	7.50	>7.50
S. aureus (Clinical isolate)	7.50	>7.50	3.75	>7.50	3.75
S. epidermidis (clinical isolate)	0.95	7.50	3.75	7.50	>7.50
Serratia marsecens (ATCC 9986)	>7.50	7.50	>7.5	7.50	>7.50
S. typhi (clinical isolate)	0.95	>7.50	3.75	>7.50	3.75
S. typhi (clinical isolate)	0.95	>7.50	>7.50	>7.50	>7.50

Table 4. MBCs of the essential oils against the bacterial organisms.

Organism	MBCs values for nine essential oils (mg/ml)				
	C. scabrida	*H. foetidum*	*A. digitata*	*E. punculatus*	*A. afra*
Acinetobacter calcoaceticus	>7.50	>7.50	>7.50	>7.50	>7.50
B. cereus (clinical isolate)	3.75	3.75	>7.50	>7.50	>7.50
E. coli (ATCC 8739)	>7.50	>7.50	>7.50	>7.50	>7.50
E. coli (clinical isolate)	>7.50	>7.50	>7.50	>7.50	>7.50
Klebsiella pneumoniae (clinical isolate)	>7.50	>7.50	>7.50	>7.50	>7.50
M. kristinae (clinical isolate)	7.50	>7.50	>7.50	>7.50	>7.50
P. aeruginosa (ATCC 7700)	7.50	3.75	>7.50	>7.50	3.75
P. vulgaris	0.95	3.75	>7.50	>7.50	>7.50
Serratia marsecens (ATCC 9986)	>7.50	>7.50	>7.50	>7.50	>7.50
S. aureus (clinical isolate)	>7.50	>7.50	3.75	>7.50	3.75
S. epidermidis (clinical isolate)	0.95	>7.50	7.50	7.50	>7.50
Streptococcus faecalis (ATCC 29212)	7.50	>7.50	>7.50	>7.50	>7.50
S. typhi (clinical isolate)	0.95	>7.50	>7.50	>7.50	>7.50
S. typhi (clinical isolate)	0.95	>7.50	>7.5	>7.50	3.75

aeruginosa cells within 3 h (Figure 1), while *A. digitata* was able to kill about 40% of *S. epidermidis* after 3 h of incubation (Figure 2). *C. scabrida* essential oils were able to kill only about 60% of the *P. aeruginosa* cells within 3 h (Figure 1) as well as *S. epidermidis* (Figure 2). But after 12 h they had all killed almost 98% of the cells of *P. aeruginosa*. The other essential oils tested were able to kill up to 90% of the cells of *S. epidermidis*, *P. aeruginosa* and *M. kristinae* after the first 3 h of contact with the oil.

Antifungal activity of the essential oils against yeast isolates

MIC of the essential oils against the yeast isolates

All the nine essential oils were tested for antifungal activities against the yeast isolates. Out of the 9 oils, *C. scabrida* and *A. afra* were the most active with MIC against 5 of the 6 yeast isolates less than 1 mg/ml (Table 5). *C. scabrida* was very active against all the five *Candida* spp. with MICs less than 1 mg/ml, while the MIC against *C. neoformans* was 7.5 mg/ml. Although, *E. punculatus* was active against 5 of the 6 yeast organisms tested, all the MICs were 7.5 mg/ml indicating weak activities. In the contrary, the MICs for *H. foetidum* were less than 1 mg/ml against most of the organisms except *Candida albicans* and *Candida parapsilosis*.

Fungicidal activity of the essential oils against the yeast isolates

Of all the essential oils evaluated for the fungicidal activity,

Table 5. MICs of the essential oils against the yeast species.

Organism	MIC of the essential oils against the yeast isolates (mg/ml)				
	C. scabrida	*H. foetidum*	*A. digitata*	*E. punculatus*	*A. afra*
C. albicans	0.06	7.50	7.50	7.50	0.12
C. glabrata	0.95	0.06	7.50	7.50	0.06
C. kruzei	0.24	0.24	7.50	7.50	0.12
C. parapsilosis	0.06	3.75	3.70	7.50	7.5
C. tropicalis	0.95	0.24	3.75	>7.50	0.24
C. neoformans	7.50	0.95	3.75	7.50	0.24

Table 6. Minimum fungicidal activity of the essential oils against the yeasts isolates.

Organism	MFC (mg/ml)				
	C. scabrida	*H. foetidum*	*A. digitata*	*E. punculatus*	*A. afra*
C. albicans	1.90	3.75	7.50	>7.50	7.50
C. glabrata	3.75	>7.5	7.50	>7.50	7.50
C. kruzei	1.90	3.75	7.50	7.50	7.50
C. parapsilosis	0.48	>7.50	7.50	7.50	7.50
C. tropicalis	>7.50	1.90	7.50	>7.50	7.50
C. neoformans	>7.50	>7.50	7.50	7.50	7.50

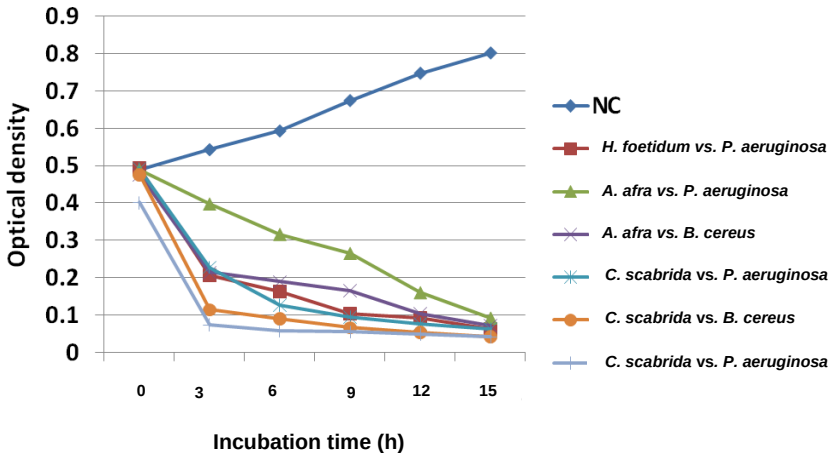

Figure 1. Killing curves of essential oils against bacterial strains showing the rate at which the essential oil killed the bacterial organisms indicated by the variation of optical density at 590 nm within different periods of time. NC = Negative control.

activity, the oils of *A. digitata* and *A. afra* were fungicidal to all the yeast isolates tested in the present study with MFC values ranging from 0.12 to 7.50 mg/ml. The essential oil from *C. scabrida* was fungicidal to 4 out of the 6 yeast isolates tested with the smallest MFC of 0.48 mg/ml against *Candida tropicalis*. Essential oils from *H. foetidum* and *E. punculatus* were active against three fungal strains out of six yeast strains tested. *C. parapsilosis* was the most susceptible to the essential oils with MFC values less than 1 mg/ml to *C. scabrida* (0.48 mg/ml) essential oils. *Candida glabrata* and *C. neoformans* were the most resistant since they were not killed by up to five essential oils and the smallest MFC obtained were 3.75 and 7.5 mg/ml, respectively. Table 6 shows the MFCs of the essential oils against the 6 yeast isolates tested.

Killing kinetics of essential oils against the yeast isolates

Two different essential oils were tested for killing kinetics against the yeast isolates based on their inhibitory and fungicidal activities in previous experiments. These were

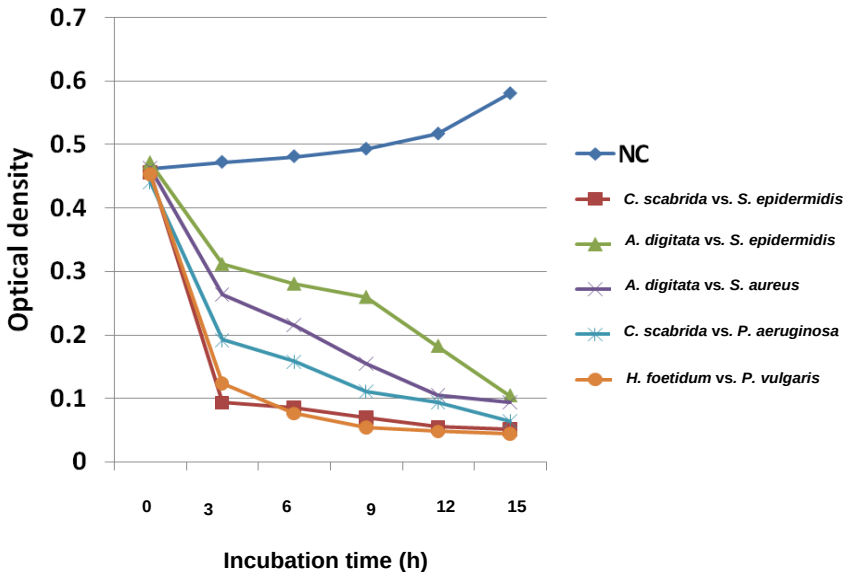

Figure 2. Killing curves of essential oils against bacterial strains indicating the rate at which the essential oil kills the bacterial strains showed by the variation of optical density at 590 nm at different periods of time. NC=Negative control.

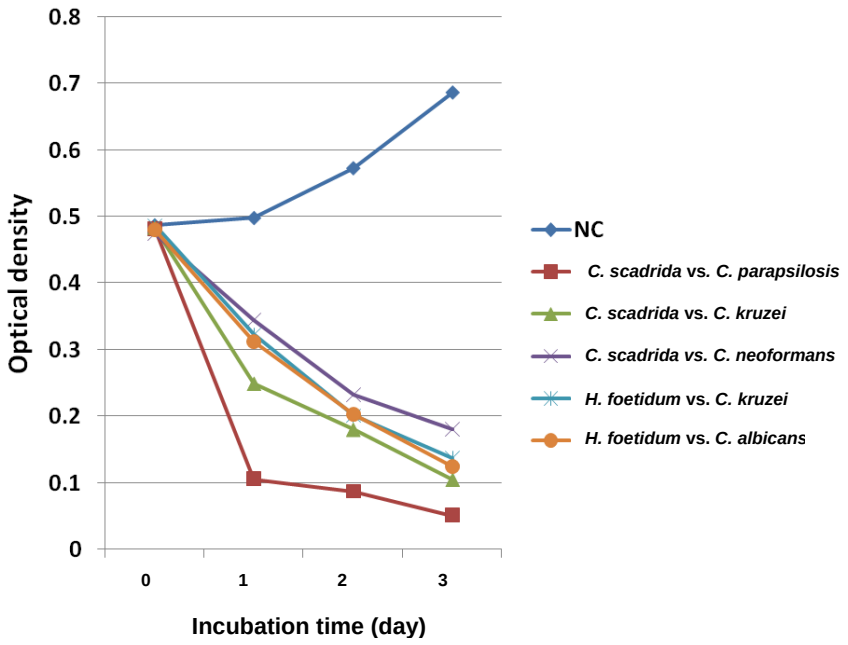

Figure 3. Killing curves of different essential oils against different yeasts isolates indicating the rate at which the essential oils were killing the fungal strains indicated by the variation of optical density at 590 nm at different time periods. NC = Negative control.

essential oils from *C. scabrida and H. foetidum.* They were tested against *C. parapsilosis, Candida kruzei, C. albicans* and *C. neoformans.*

C. scabrida essential oils were able to substantially decrease the number of yeast cells within a day (up to 90%). However, essential oils from both plants had similar activities after 2 days (Figure 3). Similarly, *C. scabrida* was more active than *H. foetidum* against *C. krusei*. *C. scabrida* essential oils were the most active against *C. parapsilopsis* and after the first day of incubation they had killed more than 90% of the cells. The negative control did not decrease the growth of the fungal cells.

DISCUSSION

Many essential oils have been known to have therapeutic and antibacterial properties, and their biological activity is currently the subject of renewed interest. Essential oils have been recognized for their therapeutic properties for centuries, however, only few of them have been characterized for their antimicrobial activities (Halcon and Milkus, 2004). Therefore, this study was set to determine the biological activity of essential oils from plants commonly used in the Southern African region against bacterial and fungal pathogens.

A. digitata commonly known as Baobab is a very common plant found in the savannas throughout the African continent. It has been used for centuries by indigenous people in the continent and even from outside the continent. It is associated with many beneficial effects. In this study, Baobab oil was able to inhibit the growth of most organisms. However, this oil had little bactericidal activity. Baobab oil is commonly used as a beauty product. It would be estimated that apart from the physiological activity of the oils on the skin, the oil might have some antimicrobial activity which will limit the growth of microorganisms such as *S. aureus* which is commonly found on the skin and can be responsible for skin infections. Fortunately, in this study, Baobab oil was bactericidal against *S. aureus* with an MBC of 3.5 mg/ml. However some studies reported that baobab oil had significant antibacterial activity since the MICs of the plant extracts were ranging from 6 to 1.5 mg/ml (Masola et al., 2009). This is in agreement with the findings of the present studies were the oil was weakly active against a number of bacterial organisms. Baobab oil was also active against *S. epidermidis*, but at higher concentrations. Previous studies by Shukla et al., (2003) indicated that the ethyl acetate and n-butanol fractions of the pericarp of *A. digitata* were found to be active against *S. aureus*, *S. epidermidis*, *Streptococcus mutans* and *P. aeruginosa*. In this study, Baobab oil was inhibitory to *P. aeruginosa*, but was not bactericidal. Studies on the antifungal activity of *A. digitata* indicated that extracts from this plant showed anti-fungal activity to a lesser extent against dermatophytes (Locher et al., 1995).

Essential oil from *A. afra* was found to be active against 4 out of 5 *Fusarium* species tested in this study. In a study by Mangena and Muyima (1999), the essential oils of *A. afra* growing in Eastern Cape, South Africa showed broad antimicrobial activities and therefore may have preservative potential for the food and cosmetic industries. The activity of the essential oil could be dependent on geographical location of the plant. A recent study has demonstrated that the composition of *A. afra* essential oil varied between the three different provinces, particularly in the levels of alpha-and beta-thujone, 1,8-cineole and camphor (Oyedeji et al., 2009). This will explain the variation of activities of plants from different areas. Their study further indicated that fresh leaves had less alpha thujone than dried leaves indicating that the oil from the fresh leaves are safer. Further studies are needed to clarify the effect of the compositional variation of the antimicrobial potential.

Essential oil from *C. scabrida* showed very good activities against all the microorganisms tested, including bacteria and fungi. Traditionally, this plant is used to treat influenza, chest and stomach afflictions, fever, diarrhea, sores, and inflammation (Watt and Breyer-Brandwijk, 1962; Scott et al., 2004). Studies in the Western Cape, South Africa showed that the aqueous infusion of *C. scabrida* showed good inhibition against *C. albicans* with a MIC value of 0.625 mg/ml (Thring et al., 2007). In East Africa, roots of *Helichrysum* species are used for eye complaints and the leaves for influenza and certain oils that are extracted from different parts of the plants are being inhaled by traditional healers to induce a trance (Reid et al., 2006). It has been reported that the chemical constituents of different essential oil support their microbial activity (Watt and Breyer-Brandwijk, 1962). *Helichrysum* spp. are used extensively in ethnomedicine in South Africa and many of the uses are associated with the treatment of infections, e.g. it is used widely for treatment of respiratory diseases. Very few studies have been conducted on extracts or essential oils from *H. foetidum*. Steenkamp et al., (2004) did not find high activities (antibacterial activity) against *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *P. aeruginosa.* In this study, the essential oil of *H. foetidum* was found to be active against *P. aeruginosa* as well as *B. cereus* and was very active against the yeast isolates. Although, no studies have been conducted on the phytochemistry of *H. foetidum* itself, studies on *Helichrysum pallasii* from Italy have indicated that the oil from this related *Helichrysum* spp. contained hexadecanoic acid (16.2%), (Z,Z)-9,12-octadecadienoic acid (6.8%), tetradecanoic acid (2.6%), and (Z)-caryophyllene (4.2%) as the main constituents of the oil from leaves (Formisano et al., 2009). Another study on related *Helichrysum* spp. in Tanzania indicated that *Helichrysum cymosum* and *Helichrysum fulgidum* contained trans-Caryophyllene, caryophyllene oxide, beta-pinene, p-cymene, spathulenol and beta-bourbonene as the main components (Bougatsos et al., 2004). Similar compounds could be found in *H. foetidum* and might be responsible for the activities observed. However, more studies are needed to identify the potential active compounds and the safety of the oil from this plant for human consumption.

Conclusion

Essential oils obtained from leaves, stems, and flowers of different plant species that are found in the Southern African region (Zimbabwe, South Africa) exhibited

antimicrobial activities, because they were able to kill or inhibit the growth of medically important bacteria and fungi used in the present study. This investigation together with previous studies provides support to the use of these essential oils as antibacterial and antifungal supplements in developing countries towards the development of new therapeutic agents. The results of this study indicate that essential oils are promising sources of natural products with potential antimicrobial activity and will guide the selection of some plant species for further pharmacological and phytochemical analysis. Additional studies both *in vitro* and *in vivo* and clinical trials would be needed to further characterize the active principles and evaluate the potential toxicity of these oils.

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