African Journal of Biotechnology Vol. 11(89), pp. 15560-15568, 6 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1444
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Antimicrobial activities of essential oils from Southern Africa against selected bacterial and fungal organisms

Samie A.[1]*, Nefefe T.[1], Gundidza M.[2], Mmbengwa V.[3] and Magwa M.[4]

[1]Department of Microbiology, Molecular Parasitology and Opportunistic Infections Program, University of Venda, Private Bag X5050, Thohoyandou, 0950 South Africa.
[2]Department of Pharmaceutical Technology, Harare Institute of Technology, P.O. Box BE 277, Ganges Road, Belvedere, Harare, Zimbabwe.
[3]School of Natural Resource Management, Nelson Mandela Metropolitan University, George Campus, Private Bag X6531, George 6530, South Africa.
[4]Department of Botany and Electron Microscope Unit, University Of Fort Harare, Private Bag X131400, Alice, 5700, South Africa.

Accepted 17 August, 2012

In the present study, essential oils from four plants including *Melissa officinalis, Mentha piperita, Pelargonium graveolens and Leucosidea sericea,* 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* spp. 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 *P. graveolens* and *M. peripeta* against bacterial and fungal strains tested in the present study 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 *P. graveolens* were fungicidal to all the yeast isolates tested in the present study with minimum fungicidal concentration (MFC) values ranging from 0.12 to 7.50 mg/ml while the essential oil from *M. piperita was* fungicidal to one of the six yeast isolates tested with the smallest MFC of 0.48 mg/ml against *Candida tropicalis*. Essential oils from *P. graveolens* were able to kill 90% of the *P. aeruginosa* cells within three hours. The present study has revealed the antimicrobial activity of *P. graveolens* and *M. piperita* and indicated 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

Infectious diseases constitute an important health problem throughout the world particularly in developing countries and contribute to about one third of all mortality (WHO, 2011). Bacterial and fungal diseases are among the most common of these infections. Following the discovery of antibiotics at the beginning of the 19th century, there was high hope for the elimination of infectious organisms however; problems hampering the

*Corresponding author. E-mail: samieamidou@yahoo.com or samie.amidou@univen.ac.za.

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

Scientific name	Common name	Source	Application
Melissa officinalis	Lemon balm	Fruits	It is used as a medicinal plant and as a seasoning herb.
Mentha piperita	Peppermint	Leaves	Peppermint oil is used mainly for flavouring toothpaste, other oral hygiene products, and chewing gum. Smaller quantities are used for flavouring confectionaries
Pelargonium graveolens	Rose geranium	Flower	Rose geranium oil has a balancing effect on the nervous system and relieves depression and anxiety, while lifting the spirits and making the world an easier place to live in. It has a balancing effect on the adrenal cortex and is great for relieving stress
Leucosidea sericea Eckl. and Zeyh	Ouhout	Leaves	Used against severe inflammation of the eyes. Also, the leaves form part of the constituents used against intestinal worm infections (Hutchings et al., 1996)

control of those infections soon emerged and include antibiotic resistance, genetic variations and the emergence and re-emergence of some infections (Iyalomhe et al., 2011). Despite the development of antibiotics, bacterial and fungal infections are still a major health threat. This is further compounded by the increase of antibiotic and drug resistant organisms. Therefore there is need for further development of new, less expensive and efficient methods for the control of these infections.

For centuries, plants have been used for the treatment of several ailments by different cultures around the world (Pirbalouti et al., 2011). The Southern African region has a rich biodiversity and local populations have been using medicinal plants for years. It is estimated that more than 70% of the population in tropical Africa including South Africa still rely on traditional medicines for their primary health care (Oni, 2010). The knowledge of the population combined to the rich biodiversity provides the perfect circumstance for the development of new drugs for the control of infectious diseases that plague the lives of many citizens of the region (Ghuman and Coopoosamy, 2011).

Essential oils (EOs) are complex mixtures of volatile secondary metabolites from plants and they are used to control various infectious diseases (Moghaddasi, 2010). Essential oils are known to have variety pharmacological effects, including anti inflammatory, antiviral and antimicrobial activities (Gundidza et al., 2009). About 3000 essential oils have been described, of which only about 10% are used in the industry and a very negligible fraction is from the Sub-Saharan Africa. Therefore, the present study was designed to determine the antimicrobial activities of essential oils from four Southern African plants traditionally used as medicine by local population.

MATERIALS AND METHODS

Preparation of essential oil

The plants used in the present study were all collected in Zimbabwe around the city of Harare between October and November, 2009. Table 1 shows a list of the plants used. 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 extracted with 2 L of water for 3 h. The oils were collected and dried under anhydrous sodium sulphate and stored at 4°C until further used.

Preparation of microorganisms

In order to evaluate the antimicrobial activity of essential oil, 20 organisms were used including gram positive and gram negative bacteria (14 in total) and yeast isolates (*Candida* spp and *Cryptococcus neoformans*). The bacterial organisms were both clinical isolates and standard isolates from ATCC (Table 2). Prior to the test, a 0.5 MacFarland standard of the bacterial cell was 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

The micro dilution method was used to determine the minimum inhibitory concentration (MIC) as previously described (Eloff, 1998) with the following modifications: a final concentration of 0.5% (v/v) Tween-20 (Sigma) was incorporated into the medium to enhance oil solubility. Briefly, 185 µl of brain heart infusion broth (Oxoid, England) (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. Brain heart infusion was prepared according to the manufacturer's instructions. 15 µl of essential oil was placed in the first well and mixed. Thereafter 100 µl of the mixture was transfered to the next well and the same procedure repeated until to the last well to achieve a serial two fold dilution in the wells. The concentrations tested varied between 0.06 mg/ml and 7.5 mg/ml. 100 µl of the microorganism's culture was added to the broth.

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

Bacteria	Origin	Type of organism
Acinetobacter calcoaceticus	Clinical isolate	G-negative
Bacillus cereus	Clinical isolate	G-positive
Escherichia coli	ATCC 8739	G-negative
Escherichia coli	Clinical isolate	G-negative
Klebsiella pneumoniae	Clinical isolate	G-negative
Micrococcus kristinae	Clinical isolate	G-negative
Proteus vulgaris	ATCC 6830	G-negative
Pseudomonas aeruginosa	ATCC 7700	G-negative
Salmonella spp	Clinical isolate	G-negative
Salmonella typhi	Clinical isolate	G-negative
Serratia marsecens	ATCC 9986	G-negative
Staphylococcus aureus	Clinical isolate	G-positive
Staphylococcus epidermidis	Clinical isolate	G-positive
Streptococcus faecalis	ATCC 29212	G-positive
Candida albicans	Clinical isolate	Yeast
Candida glabrata	Clinical isolate	Yeast
Candida kruzei	Clinical isolate	Yeast
Candida parapsilosis	Clinical isolate	Yeast
Candida tropicalis	Clinical isolate	Yeast
Cryptococcus neoformans	Clinical isolate	Yeast

The plates were then incubated at 37°C for 24 h. At the end of the incubation period, 50 µl of iodo-nitro tetrazolium (INT) was added to each well to indicate the presence of living cells in the wells by a change of the color from clear to brown. The minimum inhibitory concentration 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 EOs were used to determine the MBC. The wells in plates that showed no visible growth were inoculated onto 90 mm Mueller Hinton agar plates and 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 EO 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 contained 300 µl.

Each test was run in two different wells. After every 3 h, 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 two 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 1 ml of a 1 McFarland standard of the organisms grown in brain heart infusion broth (Apak and Olila, 2006). Afterward, six 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 to give a concentration varying from 7.5 to 0.06 mg/ml. Sterile dimethylsulfoxide (DMSO) was used as negative control and nystatin was used as positive control.

The plates were then incubated at 30°C for three days. After three days, the radial zone of inhibition was measured by using a ruler and the diameter of inhibition zone was 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 plates as described above. The Petri dishes were marked according to the number of wells and EO as appearing on the microtitre plates.

The plates were incubated at 30°C for two days. The smallest concentration that did not show any growth on the agar plates was regarded as the MFC.

Table 3. Minimum inhibitory concentration of the essential oils against the bacterial organisms (mg/ml).

Microorganism	*P. graveolens*	*M. officinalis*	*M. piperita*	*L. sericea*
A. calcoaceticus (clinical isolate)	>7.50	>7.50	0.95	>7.50
B. cereus (clinical isolate)	0.95	>7.50	>7.50	>7.50
E. coli (ATCC 8739)	>7.50	7.50	7.50	>7.50
E. coli (clinical isolate)	>7.50	7.50	>7.50	>7.50
K. pneumoniae (clinical isolate)	>7.50	7.50	0.95	>7.50
M. kristinae (clinical isolate)	7.50	7.50	7.50	>7.50
P. aeruginosa ATCC 7700	3.75	>7.50	7.50	>7.50
P. vulgaris (clinical isolate)	7.50	3.75	7.50	>7.50
S. faecalis (ATCC 29212	7.50	>7.5	0.95	>7.50
S. aureus (clinical isolate)	3.75	>7.50	>7.50	>7.50
S. epidermidis (clinical isolate)	7.50	3.75	0.95	>7.50
S. marsecens (ATCC 9986)	7.50	>7.50	7.50	>7.50
S. typhi (clinical isolate)	>7.50	7.50	7.50	>7.50
S. typhi (clinical isolate)	>7.50	7.50	7.50	>7.50

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 sterile fresh brain heart infusion broth was added to the wells together with 100 µl of the fungal culture. 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 transfered to a new microtitre plate with 200 µl of sterile distilled water and the OD was read using ELISA reader every day for six 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. The Chi square was used and the 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 the four plant species tested, the oil of *Melissa piperita* was the most active with MIC varying from 0.95 to 7.50 mg/ml against 11 of the bacterial strains used in the present study. *M. piperita* gave an MIC less than 1 mg/ml against four bacterial strains while *Pelargonium graveolens* gave an MIC of less than 1 mg/ml against one isolate. Table 3 shows the minimum inhibitory concentrations of the essential oils against the bacterial organisms. Essential oils of *P. graveolens* and *Melissa officinalis* were active against eight pathogenic bacterial strains with the MIC values ranging from 0.95 to 7.5 mg/ml. The essential oil from *Leucosidea cericea* was not active against all the bacterial strains tested in this study at the concentrations used.

Of all the organisms tested, the *E. coli* strains both the standard ATCC strain and the clinical strain were the most resistant to the essential oils while *M. kristinae* (clinical isolate) and *P. aeruginosa* ATCC 7700 were the most susceptible with MIC more than 7.5 mg/ml only for *L. cericea* against *M. kristinae* and *M. officinalis* and *L. cericea* against *P. aeruginosa*. Both strains of *Salmonella typhi* (clinical isolates) had similar resistance profiles to the essential oils.

Bactericidal activity of the essential oils

Of all the four essential oils tested for the bactericidal activity, *L. cericea* and *M. officinalis* were not bactericidal to any of the organisms tested (Table 4). *M. piperita* gave an MBC less than 1 mg/ml only against one bacterial strain that was *S. epidermidis* clinical isolate. The essential oil of *P. graveolens* was active against five different bacterial with no MBC less than 1 mg/ml. However out of all the essential oils tested, only two essential oils, that of *M. officinalis* and *L. cericiae* were not bactericidal against any of the bacterial strains tested in the present study. *S. epidermidis* was the most susceptible organism to the essential oils as it showed MBC values less than 1 mg/ml to the essential oils from *M. piperita*

Killing kinetics of the bacterial organisms by the essential oils

The two plant species (*P. graveolens* and *M. piperita*), that showed MBC values less than 7.5 mg/ml

Table 4. Minimum bactericidal concentrations of the essential oils against the bacterial organisms (mg/ml).

Organism	*P. graveolens*	*M. officinalis*	*M. piperita*	*L. sericea*
A. calcoaceticus (Clinical isolate)	7.50	>7.50	3.75	>7.50
B. cereus (Clinical isolate)	3.75	>7.50	>7.50	>7.50
E. coli (ATCC 8739)	>7.50	>7.50	>7.50	>7.50
E. coli (clinical isolate)	>7.50	>7.50	>7.50	>7.50
K. pneumoniae (clinical isolate)	>7.50	>7.50	3.75	>7.50
M. kristinae (Clinical isolate)	>7.50	>7.50	7.50	>7.50
P. aeruginosa ATCC 7700	3.75	>7.50	>7.50	>7.50
P. vulgaris (Clinical isolate)	>7.50	>7.50	7.50	>7.50
S. marsecens (ATCC 9986)	>7.50	>7.50	>7.50	>7.50
S. aureus (Clinical isolate	3.75	>7.50	>7.50	>7.50
S. epidermidis (clinical isolate)	>7.50	>.750	0.95	>7.50
S. faecalis (ATCC 29212	7.50	>7.50	3.75	>7.50
S. typhi (clinical isolate)	>7.50	>7.50	7.50	>7.50
S. typhi (Clinical isolate)	>7.50	>7.50	>7.50	>7.50

significantly reduced 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 (Figure 1). *P. graveolens* oil was able to kill 90% of the *S. epidermidis* cells within three hours (Figure 1). However, it was able to kill only about 60% of the *P. aeruginosa* cells within 3 h, but after 12 h they had killed almost 78% of the cells of *P. aeruginosa*.

Antifungal activity of the essential oils against yeast isolates

All the essential oils were tested for antifungal activities against the yeast isolates. *M. officinalis* and *L. cericea* were not active against all the yeast isolates tested while all the MICs for *P. graveolens* were less than 1 mg/ml against all the organisms (Table 5).

Fungicidal activity of the essential oils against the yeast isolates

Of all the essential oils evaluated for the fungicidal activity, the oils of *P. graveolens* was fungicidal to all the yeast isolates tested with MFC values ranging from 0.12 to 7.50 mg/ml. The essential oils from *M. officinalis* and *L. ceriiciae* were not fungicidal to any of the yeast isolates tested. *C. parasilopsis* was the most susceptible to the essential oils with MFC values less than 1 mg/ml to the essential oils from *P. graveolens* (0.12 mg/ml). *Candida glabrata* and *Candida krusei* were the most resistant since they had the highest MFC obtained at 7.5 mg/ml.

Table 6 shows the minimum fungicidal concentrations of the essential oils against the 6 yeast isolates tested.

Killing kinetics of essential oils against the yeast isolates

Three different essential oils were tested for killing kinetics against the yeast isolates based on their inhibitory and fungicidal activities in previous experiments. *P. graveolens* showed the most activity against most of the fungal organisms tested. The essential oil from *P. graveolens* was able to kill only about 40% of *C. albicans* cells after the first day. *P. graveolens* essential oil was most active against *C. parapsilopsis* followed by *C. krusei* (Figure 2). *M. piperita* showed less activity against *C. parapsilopsis* and was not able to completely kill the cells after three days of exposure.

DISCUSSION

Many essential oils are known to have therapeutic and antibacterial properties, and their biological activity is currently the subject of renewed interest (Okigbo et al., 2008). 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. Of the essential oils tested, *P. graveolens* and *M. piperita*

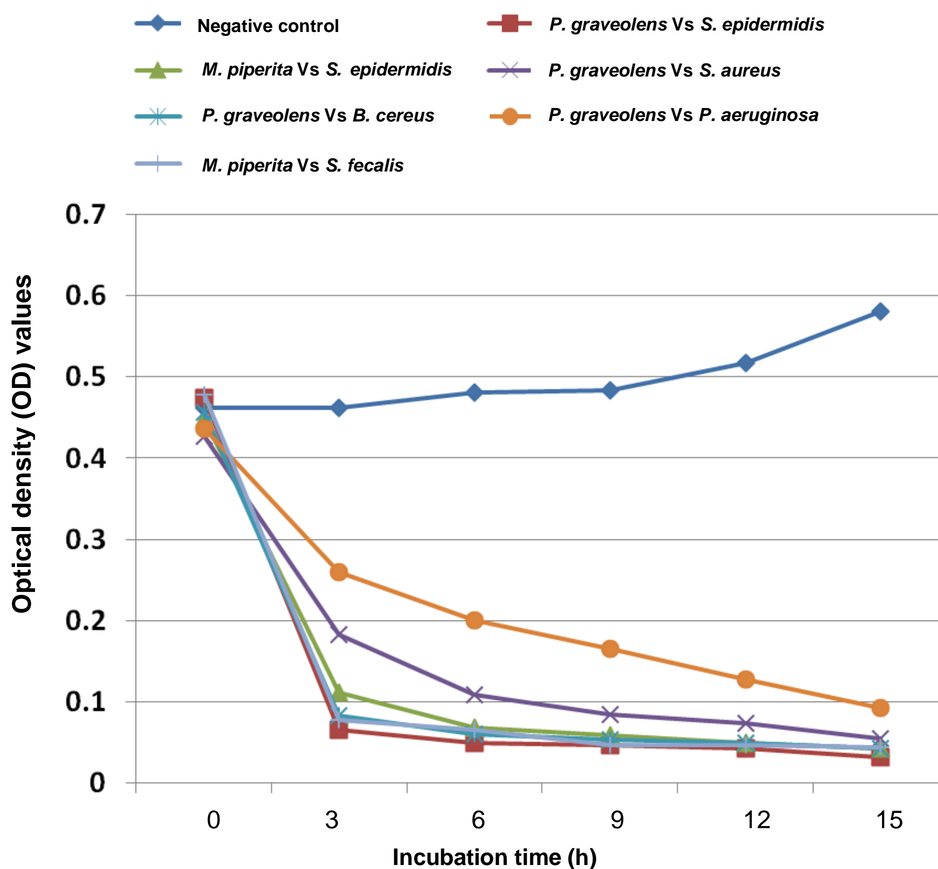

Figure 1. Killing curves of essential oils against bacterial strains showing the rate at which the essential oil killed the bacterial organisms indicated by the variation of optical density at 590 nm at different periods of time.

Table 5. Minimum inhibitory concentrations of the essential oils against the yeast species (mg/ml).

Name of organism	*P. graveolens*	*M. officinalis*	*M. piperita*	*L. sericea*
C. albicans	0.24	>7.50	1.90	>7.50
C. glabrata	0.12	>7.50	1.90	>7.50
C. kruzei	0.06	>7.50	>7.5	>7.50
C. parapsilosis	0.06	>7.50	3.75	>7.50
C. tropicalis	0.24	>7.50	3.75	>7.50
C. neoformans	0.24	>7.50	3.75	>7.50

Table 6. Minimum fungicidal concentrations of the essential oils against the yeasts isolates (mg/ml).

Name of organism	*P. graveolens*	*M. officinalis*	*M. piperita*	*L. sericea*
C. albicans	3.75	>7.50	>7.50	>7.50
C. glabrata	7.50	>7.50	>7.50	>7.50
C. kruzei	7.50	>7.50	>7.50	>7.50
C. parapsilosis	0.12	>7.50	7.50	>7.50
C. tropicalis	1.90	>7.50	>7.50	>7.50
C. neoformans	3.75	>7.50	>7.50	>7.50

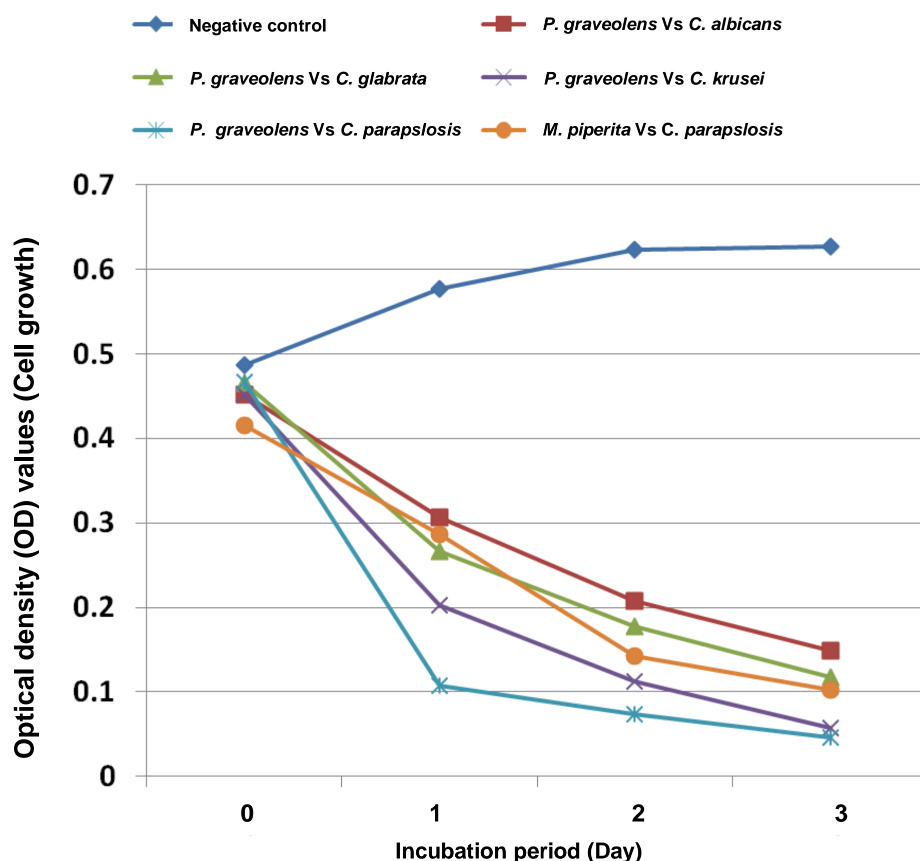

Figure 2. Killing curves of essential oils against yeast isolates.

showed consistent antimicrobial activities.

Mentha piperita essential oil exhibited weak activity against some organisms such as the gram negative bacterial organism *E. coli*. It was inhibitory to all the yeast isolates tested; however, it was not fungicidal to any of the six yeast pathogens. In a previous study by Mimica-Dukic et al. (2004), the essential oil from *M. piperita* was active against *Trichophyton tonsurans* and *C. albicans* with low MIC (8 µl/ml) of *M. piperita* oil for *E. coli.* This study also indicated that *M. pip*erita essential oil exhibited the highest OH radical scavenging activity, reducing OH radical generation in the Fenton reaction by 24% (pure oil). Hammer et al. (1999) reported cidal activity of *M. piperita* oil at 0.25% (v/v) equivalent to 25 µl/ml for *E. coli* and *C. albicans* and 12 µl/ml for *Staphylococcus aureus*. *M. piperita* oil was also reported to be fungicidal to *C. albicans* at 500 ppm (Tampieri et al., 2005). In another study by Aridogan et al. (2002), *M. piperita* antimicrobial activity was recorded only to *S. aureus* and not to *E. coli* (Aridogan et al., 2002). These differences could be due to a difference in chemical composition of the oils collected from different parts of the world. A study on the plants from Iran indicated that Menthanol (36.24%) and menthone (32.42%) were the major compounds of the *M. piperita* essential oil (Behnam et al., 2006). Menthol has been reported to be responsible for the antimicrobial activity of *M. piperita* (Barrera-Necha et al., 2009). Other studies revealed higher antimicrobial property of *M. piperita* essential oil with menthol concentration as low as 3.6%. Further studies are needed in order to identify the active compounds from this plant growing in the Southern African region and to associate the presence of these compounds to the biological effect observed.

In the present study, the essential oils from *M. officinalis* and *L. cericea* were not active against most microorganisms tested. Although several studies have been conducted on M. *officinalis*, very few studies have been conducted on *L. sericea*. *Leucosidea sericiea* also known as the Oldwood' tree is the sole representative of the genus *Leucosidea* and its use in traditional medicine by some of the indigenous people of the Southern African region has been known for over a hundred years. Compounds such as the known cholestane triterpenoids β-sitosterol and β-sitostenone have been isolated from stems of this plant (Nair et al., 2012). In a recent study by Aremu et al. (2010), the leaf extracts of *L. sericea* exhibited broad spectrum antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* ranging from

0.025 to 6.25 mg/ml and contained higher amounts of phenolic compounds including alkaloids and saponins. *M. officinalis* essential oil has been shown to have good antioxidant effect (Marongiu et al., 2004) as well as antiviral effect (Schnitzler et al., 2008). Cytotoxicity studies of essential oils *M. officinalis*, have shown that the oil from this plant was highly toxic to *Spodoptera littoralis* larvae with LD_{50} < or = 0.05 microl/larvae (Pavela, 2005). Therefore it is important to verify the potential toxic effect of this oil before advising its use by human. Friedman et al. (2004) studied the antibacterial activities of *M. officinalis* essential oil and found that it was not effective against *E. coli* in juice. In the contrary, Mimica-Dukic et al. (2004) indicated that the most effective antibacterial activity of *M. officinalis* essential oil was expressed on a multiresistant strain of *Shigella sonei* and a significant rate of antifungal activity was exhibited on *Trichophyton* species. We did not test the activity of the oil against these two organisms. Studies by Bosman et al. (2004) indicated that the petroleum extract of the leaves of this plant had antimicrobial activity against *S. aureus*, *B. subtilis* and *C. albicans.* Bioassay-guided fractionation, by the same authors, of the leaves and flowers extracts yielded the phloroglucinol derivatives, aspidinol and desaspidinol.

Essential oil of *P. graveolens* exhibited absolute fungitoxicity against the toxigenic strains of *A. flavus* with MIC of 0.75 g L^{-1} and exhibited a fungistatic nature (Singh et al., 2008). The oil also showed excellent anti-aflatoxigenic efficacy as it completely inhibited aflatoxin B_1 production even at 0.50 g L^{-1}. Jeon et al. (2009) studied the acaricidal activities of compounds derived from the oil of *P. graveolens* leaves against *Tyrophagus putrescentiae* and discovered that the toxic compounds against the food mite were geraniol (1.95 microg/cm3), followed by nerol (2.21 microg/cm3), citral (9.65 microg/cm3), benzyl benzoate (11.27 microg/cm3), and beta-citronellol (15.86 microg/cm3). In the present study, the essential oil of *P. graveolens* showed excellent activities against all the microorganisms tested including bacteria and yeast. In a study by Rosato et al. (2008), *P. graveolens* essential oil was the most effective in combination with amphotericin B in inhibiting all the *Candida* species evaluated. This indicates that *P. graveolens* essential oil can be used for the control of fungal pathogens both alone or in combination with purified drugs such as amphotericin B. The activities observed could be due to the compounds previously identified such as geraniol or beta citronellol all identified in the essential oil of *P. graveolens* with anti mites activity.

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. The present 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 essential oil from *P. graveolens* showed good antibacterial and anti-fungal activities and could be used in 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.

ACKNOWLEDGEMENTS

The authors are very grateful for the financial support obtained from the National Research Foundation, South Africa and the Research and Publication committee of the University of Venda.

REFERENCES

Apak L, Olila D (2006). The *in-vitro* antibacterial activity of *Annona senegalensis , Securidacca longipendiculata* and *Steganotaenia araliacea* - Ugandan medicinal plants. Afr. Health Sci. 6(1): 31-35.

Aremu AO, Fawole OA, Chukwujekwu JC, Light ME, Finnie JF, Van Staden J (2010). *In vitro* antimicrobial, anthelmintic and cyclooxygenase-inhibitory activities and phytochemical analysis of *Leucosidea sericea*. J. Ethnopharmacol. 131 (1): 22-27.

Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E (2002). Antimicrobial activity and chemical composition of some essential oils. Arch. Pharm. Res., 25: 860-864.

Barrera-Necha L.L, Garduno-Pizana C, and Garcia-Barrera L.J (2009). *In vitro* antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) snyder and hansen. Pak. J. Nutr., 8: 17-21.

Behnam S, Farzaneh M, Ahmadzadeh M, Tehrani AS (2006). Composition and antifungal activity of essential oils of *Mentha piperita* and *Lavendula angustifolia* on post-harvest phytopathogens. Comm. Agric. Appl. Biol. Sci., 71(3): 1321-1326.

Bosman AA, Combrinck S, Roux-van der Merwe R, Botha BM, McCrindle RI (2004). Isolation of an antihelmintic compound from *Leucosidea sericea*. S. Afr. J. Bot., 70(4): 509–-511.

Eloff, J.N., (1998). A sensitivity and quick micro plate method to determine the minimal inhibitory concentration of plants extracts for bacteria. Planta Med., 64:711-713.

Friedman M, Henika PR, Levin CE, Mandrell RE (2004). Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. J. Agric. Food Chem., 52(19): 6042-6048.

Ghuman S, and Coopoosamy RM (2011). Crude sample preparation, extraction and *in vitro* screening for antimicrobial activity of selected wound healing medicinal plants in KwaZulu-Natal, South Africa: A review. J. Med. Plant. Res. 5(16): 3572-3576.

Gundidza M, Gweru N, Magwa ML, Mmbengwa V, Samie A (2009). The chemical composition and biological activities of essential oil from the fresh leaves of *Schinus terebinthifolius* from Zimbabwe. Afr. J. Biotechnol. 8(24):7164-7169.

Halcon L, Milkus K (2004) *Staphylococcus aureus* and wounds: a review of tea tree oil as a promising antimicrobial. Am. J. Infect. Control 32:402-408.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86:985-990.

Iyalomhe GBS, Iyalomhe SI, Eholor RE (2011). Antibiotic prescription and resistance: A contemporary literature review. Int. J. Med. Med. Sci. 3(14):376-380

Jeon JH, Lee CH, Lee HS (2009). Food protective effect of geraniol and its congeners against stored food mites. J. Food Prot. 72(7):1468-1471.

Marongiu B, Porcedda S, Piras A, Rosa A, Deiana M, Dessì MA (2004). Antioxidant activity of supercritical extract of *Melissa officinalis* subsp. officinalis and *Melissa officinalis* subsp. inodora. Phytother. Res. 18(10):789-792.

Mimica-Dukic N, Bozin B, Sokovic M, Simin N (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J. Agric. Food Chem. 52(9):2485-2489.

Moghaddasi MS (2010). Saffron chemicals and medicine usage. J. Med. Plants Res. 4(6):427-430.

Motsei ML, Lindsey KL, Van staden JAK (2003). Screening of traditionally used South Africa plants for antifungal activity against *Candida albicans*. J. Ethnopharmacol. 86:235-241.

Nair JJ, Aremu AO, Van Staden J (2012). Anti-inflammatory effects of *Leucosidea sericea* (Rosaceae) and identification of the active constituents. S. Afr. J. Bot. 80:75-76.

Okigbo RN, Eme UE, Ogbogu S (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnol. Mol. Biol. Rev. 3(6):127-134.

Oni PI (2010). Ethnobotanical survey of a fallow plot for medicinal plants diversity in Idena village Ijebu-Ode, South-western Nigeria. J. Med. Plant. Res. 4(7):509-516.

Pavela R (2005). Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. Fitoterapia 76(7-8):691-696.

Pirbalouti AG, Hamedi B, Abdizadeh R, Malekpoor F (2011). Antifungal activity of the essential oil of Iranian medicinal plants. J. Med. Plant. Res. 5(20):5089-5093.

Rosato A, Vitali C, Gallo D, Balenzano L, Mallamaci R (2008). The inhibition of *Candida* species by selected essential oils and their synergism with amphotericin B. Phytomedicine 15(8):635-638.

Samie A, Obi CL, Lall N, Meyer JJM (2009). *In vitro* cytotoxicity and antimicrobial activities, against clinical isolates of *Campylobacter* species and *Entamoeba histolytica*, of local medicinal plants from the Venda region, in South Africa. Ann. Trop. Med. Parasitol. 103(2):159-170.

Schnitzler P, Schuhmacher A, Astani A, Reichling J (2008). *Melissa officinalis* oil affects infectivity of enveloped herpes viruses. Phytomedicine 15(9):734-740.

Singh P, Srivastava B, Kumar A, Kumar R, Dubey NK, Gupta R (2008). Assessment of *Pelargonium graveolens* oil as plant-based antimicrobial and aflatoxin suppressor in food preservation. J. Sci. Food Agric. 88(14):2421-2425.

Tampieri MP, Galuppi R, Macchioni F, Carelle MS, Falcioni L, Cioni PL, Morelli I (2005). The inhibition of *Candida albicans* by selected essential oils and their major components. Mycopathology 159(3):339-345.

World health statistics (WHO) (2011). Geneva, Switzerland, ISBN 978 92 4 156419 9.

Yaya R, Jae-soek S, Jae-kwan H (2008). Screening of Thai medicinal plants for antibacterial activity. J. Biotechnol. 51:308-312.