Research Article
ISSN: 0974-6943

P.Sivamani et al. / Journal of Pharmacy Research 2010, 3(5),1049-1055

Available online through
www.jpronline.info

In vitro antifungal activity of essential oils of selected herbals against isolates from HIV/ AIDS patients

P.Sivamani[1]*, R.Sumathi[2] ,V.Thiagarajan[3] G.Singaravelu[1]

[1]*Department of Zoology, Thiruvalluvar University, Vellore, Tamilnadu, India.*

[2]*Department of Microbiology & Nanotechnology, Microlabs, Institute of Research and Technology, Arcot, Vellore Dt, Tamilnadu, India,*

[3] *Centre for Advanced Studies in Botany, University of Madras,Guindy Campus, Chennai, India*

Received on: 21-01-2010; Revised on: 16-03-2010; Accepted on:17-04-2010

ABSTRACT

Opportunistic infections are common and in the cases of HIV/AIDS they are very harmful. Fungi isolated from HIV/AIDS positive patients were tested for their sensitivity to essential oils from *Cymbopogon citratus, Cymbopogon martinii, Cinnamomum zeylanicum, Rosmarinus officinalis, Mentha piperita, Pelarogonium graveolens,* and *Vitex negundo.* In agar well diffusion method the selected essential oils were effective against all forms such as yeast, yeast like fungi and molds and mostly comparable to the standard reference antifungal drug miconazole (100 µg). The minimal inhibitory concentration (MIC) of *C. citratus* and *C.martinii* were effective but to the least level, when compared with the reference drug. The MIC of *C.zeylanicum* was best and very much comparable to the reference drug. The MIC of *R.officinalis* was variable from more effective to least effective against different test organisms. The MIC of *M. piperita was* effective but to the least level (*1000* µg/ml), when compared with the reference drug. The MIC of *P.graveolens,* was effective but to the least level (500 µg/ml), when compared with the reference drug. The MIC of *Vitex negundo* was effective but to the least level (500 µg/ml), when compared with the reference drug.The results for minimum fungicidal concentration (MFC) were similar to MIC results, but in MBC confirmation was made by the absence of visible growth in culture media. The antifungal activity is attributed to the components of essential oils, which mostly interferes with the cell membrane structure and function. According to the results of this study, the essential oil or their ethanol extract of those essential oils may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of fungal diseases in various patients, particularly, for HIV/AIDS patients.

Key words: Antifungal activity, opportunistic fungi, HIV/AIDS, essential oils, aromatherapy,

INTRODUCTION

Before the widespread use of potent combination antiretroviral therapy (ART), opportunistic infections (OIs), which have been defined as infections that are more frequent or more severe because of immunosuppression in HIV-infected persons, were the principal cause of morbidity and mortality in this population. In the early 1990s, the use of chemoprophylaxis, immunization, and better strategies for managing acute OIs contributed to improved quality of life and improved survival (1). Although hospitalizations and deaths have decreased since the implementation of ART, OIs remain a leading cause of morbidity and mortality in HIV-infected persons (2)(3)(4)(5)(6)(7)(8)(9)(10).

In recent years there has been an increasing interest in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Essential oils, odors and volatile products of plant secondary metabolism, have a wide application in folk medicine as well as in fragrance industries. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro- or steam-distillation. The main constituents of essential oils, for example, monoterpenes and sesquiterpenes and phenylpropanoids including carbohydrates, alcohols, ethers, aldehydes and ketones, are responsible for the fragrant and biological properties of aromatic and medicinal plants (11) Various essential oils and their components possess pharmacological effects, demonstrating anti-inflammatory, antioxidant and anti-cancerogenic properties (12)(13)(14). In addition to inducing resistance, antibiotics are sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions (15). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases (16) (17).

It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (18). Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (19)(20)(21)(22)(23). All the oils tested exhibited different degrees of antifungal activity against *A. fumigatus* and *A. niger*. The maximum antimycotic activity was shown by *C. martinii* followed by *C. citratus*, *Eucalyptus globulus* and *C.zeylenicum.* Aggarwal, *et al*. (24) reported antimycotic activity of *C. martinii* against *A. niger*. The oil of *C. citratus* was effective against fungal pathogens causing diseases in plants and human beings (25). Quale, *et al*. (26) treated infections caused by

***Corresponding author.**
P.SIVAMANI *M.Sc., M.Phil.,*
Department of Zoology,
Thiruvalluvar University, Vellore,
Tamilnadu,India,PIN- 632 004
Tel.: + 91-09944969596
E-mail:sivamnee65@gmail.com

Candida in AIDS patients with a drug based on Cinnamon. In our study we also found that essential oil extracted from *C. zeylanicum* demonstrated strong antifungal activity on both the species of *Aspergillus.* The antimycotic activity of cinnamon bark due to presence of cinnamaldehyde is well known (27). Similarly, *in vitro* antimicrobial activity of *C.zelyanicum* (bark) against human pathogenic fungi and commensally bacteria was studied by (28) and (29). The oils of *M.spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The essential oil of mint was found to have strong antimycotic activity against *C. albicans.*(30).

The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS)(31), as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food. Such antimicrobial activity is due to the presence of bioactive substances such as flavonoids, terpenes, coumarines and carotenes(32). The objective of this work was to study the effect of the essential oils of Lemongrass oil, Palmarosa oil, Cinnamon bark oil, Rosemary oil, Geranium oil, Peppermint oil, and Chaste tree leaf oil on the growth of fungus commonly associated with opportunistic infections in HIV/ AIDS patients.

MATERIALS AND METHODS

Essential oils

Seven essential oils such as Lemongrass oil (*Cymbopogon citratus*-Graminae), Palmarosa oil (*Cymbopogon martini*-Graminae), Cinnamon bark oil (*Cinnamomum zeylanicum*-Lauraceae), Rosemary oil (*Rosmarinus officinalis*-Labiatae), Geranium oil (*Pelargonium graveolens*-Geraniaceae), Peppermint oil (*Mentha piperita*-Labiatae), and Chaste tree leaf oil (*Vitex negundo*-Lamiaceae) were obtained from Aromax Trading Co, Chennai, Tamilnadu, India (commercial producers of plant essential oils and aromatic substances) were used in this study. Quality of the oils was ascertained to be more than 98% pure. The oil was stored in the dark at 4°C until used within a maximum period of one week.

Test fungi

Microorganisms (Clinical bacterial isolates from HIV infected) such as *Candida albicans Candida tropicalis Cryptococcus neoformans Aspergillus nige, Aspergillus flavus, Aspergillus fumigatu, Penicillium Sp, Mucor Spp, Rhizopus Spp* were obtained from the Microlabs, Institute of research and technology, Arcot, Vellore district, Tamilnadu, India. Sabouraud's dextrose agar (SDA) and broth (SDB) were used for culturing of test fungi. All strains were stored at 20°C in the appropriate medium containing 10% glycerol and regenerated twice before use.

Qualitative chemical analysis of essential oils

The essential oils were subjected to qualitative chemical analysis for secondary metabolites, tannins, saponins, steroid, alkaloids, phenolics, terpenes and glycosides in accordance with (33) and (34)Harborne with little modification (35) and (36).

Antifungal activity

Agar well diffusion method

In this study standard agar well diffusion method was followed (37) (38) (39) (40). Each fungal isolate was suspended in Sabouraud's Dextrose (Himedia, India) broth and diluted to approximately 10^5 colony forming unit (CFU) per mL. They were "flood-inoculated" onto the surface of Sabouraud's Dextrose agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 100µl of the samples solutions were delivered into the wells. The plates were incubated for 48 h at Room Temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Ethanol was used as solvent control. Miconazole was used as reference antibacterial agent. The tests were carried out in duplicates.

Minimum inhibitory concentration (MIC) and Minimal fungicidal concentration (MFC)

Antifungal activity was measured using a dilution in agar technique (41). The essential oil (100 mg) was solubilized in 1 ml of dimethyl sulfoxide (DMSO) and serially two fold diluted in Yeast Nitrogen Base Phosphate (YNBP) broth (Merck, Germany) to obtain a concentration range of 15.6-1000 µl/ml. YNBP broth containing only DMSO diluted in the same way, which did not influence fungal growth, were included as controls. All fungal strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an OD (530 nm) of 0.05 (equivalent to 1 X 10^6 CFU/ml). This suspension was used as the inoculum for the test in the agar plates. Fungal suspensions (3µl) were inoculate using a automatic micropippete (Transasia), and plates (diameter: 25 cm) were incubated at 37°C for 48 h. the minimal inhibitory concentration (MIC) was defined as the minimal concentration of the essential oil which completely inhibited the visible growth of the fungus. and MFC as the lowest concentration that completely inhibited fungal growth in plate. An antifungal agent miconazole (100 µg) included as reference antifungal agent, was tested using the same technique. All antifungal assays were tested in duplicate.

Statistical analysis

Data were analyzed using Least Significant Difference (LSD) test following one way analysis of variance (ANOVA) using SPSS 10.0 computer software package. Difference on statistical analysis of data were considered significant at $p<0.05$.

RESULTS:

Results for qualitative chemical analysis essential oils

Alcohols, terpenoids, phenolic compounds, flavanoids, alkaloids, aldehydes were identified in essential oils all four such as lemongrass oil, palmarosa oil, cinnamon bark oil and rosemary. Geranium oil showed positive results for alcohol, terpenoids and alkaloids. In peppermint oil, alcohols, terpenoids, flavanoids and alkaloids were observed. Chaste tree leaf oil was found to possess phenolics, terpenoids, flavanoids and alkaloids.

Results for agar well diffusion method

In agar well diffusion method the selected essential oils were effective against all forms such as yeast, yeast like fungi and molds. *C. citratus* was highly active against *Mucor spp(*14.25±0.97), *C.neoformans(* 13.0±0.51) and least against *A.flavus spp*. (8.13±0.636) *C.martinii* was highly active against *C.albicans*(13.25±0.90), *C.tropicalis*, (13.16±0.53),*C.neoformans (* 13.89±0.43) and least against *A.flavus* (11.03±0.48). *C.zeylanicum* was highly active against *C.neoformans* (21.83±0.74) *A.niger* (21.15±0.86) *C.albicans*, (19.23±0.80) and least against *Mucor spp* (11.87±0.77). *R. officinalis* was highly active against *Mucor spp(14.8*±0.70), *C.albicans* (13.12±0.80) and least against. *A.flavus* (7.97±0.73). *M.piperita* was highly active against *Rhizopus spp* (21.06±0.67),*C.albicans* (19.40±0.73) and least against *A.fumigatus*(*8.11*±0.37). *P. graveolens* was highly active against *Rhizopus spp* (18.04±0.55) *Mucor spp*, (17.51±0.96) and least against *A.niger (*9.03±0.58). *V.negundo* was highly active against *Rhizopus spp* (13.09±0.43),*A.niger* (13.03±0.81), *C.albicans* (12.09±0.59) and least against *C.tropicalis* (8.99±0.47). All fungi were found to be sensitive to all test essential oils and mostly comparable to the standard reference antifungal drug miconazole (100 µg).

Results for minimum inhibitory concentration (MIC) of essential oils

The minimal inhibitory concentration of *C. citratus* was 250 µg/ml *against C.albicans C.tropicalis* and *C.neoformans* and it was 500 µg/ml, for *A.niger, A.flavus, A.fumigatus Mucor spp* and *Rhizopus spp*. The results showed that they effective but to the least level, when compared with the reference drug miconazole. The minimal inhibitory concentration of *C. martinii* was similar to *C. citratus*.

The minimal inhibitory concentration of *C.zeylanicum* was 15.6 µg/ml against *C.albicans, C.tropicalis*, and *C.neoformans and* 31.25 µg/ml *against A.niger, A.flavus, A.fumigatus, Mucor spp*, and *Rhizopus spp*.. The results were best and very much comparable to the reference drug.

The minimal inhibitory concentration of *R.officinalis* was 125 µg/ml against *C.albicans* and *C.tropicalis*, and it was 250 µg/ml *C.neoformans* and 500 µg/ml for *A.niger A.flavus, A.fumigatus Mucor spp* and *Rhizopus spp.* The results showed that they were active against *C.albicans* and *C.tropicalis* effective; moderate to *C.neoformans* but to the least level against other organisms, when compared with the reference drug.

The minimal inhibitory concentration of *M. piperita* was 1000 µg/ml for all the test fungi. The minimal inhibitory concentration of *P.graveolens*, was 500 µg/ml for all the test fungi. The minimal inhibitory concentration of *Vitex negundo* was 500 µg/ml for all the test fungi. The results showed that they were effective but to the least level, when compared with the reference drug.

Results for Minimum fungicidal concentration (MFC) of essential oils

The results for minimum fungicidal concentration (MFC) were similar to minimum inhibitory concentration (MIC) results, but in MBC confirmation was made by the absence of visible growth in culture media.

DISCUSSION

It is estimated that every minute, five people between the ages of 10 and 24 become infected with HIV somewhere in the world (42). Because HIV does not have to be reported in many states, the estimate is conservative (43). Approximately 50% of people with AIDS or HIV are using complementary and alternative modalities (CAMs) (44). Initially, HIV-positive or AIDS-infected people used CAM therapies that were thought to have immunostimulatory or antiviral properties (45). However, as antiretroviral drugs became more successful, patients began choosing to add CAMs to their conventional regimes for specific symptom relief (46). Although aromatherapy is a lesser known complementary therapy, it has much to offer nursing care of HIV/AIDS in particular for the control of resistant infections (47).

While the recognized definition states that "aromatherapy is the use of essential oils for therapeutic purposes" (48), the definition of clinical aromatherapy (as used in nursing) is more specific: "The use of essential oils for outcomes that are measurable" (49). The definition of essential oils is also very specific: "Essential oils are the steam distillate of aromatic plants" (50). Other kinds of extracts that are not obtained by steam distillation are not essential oils. Extracts may contain residues of allergenic solvents.

Lemongrass (*Cymbopogon citratus*) was found to be as effective in a 2.5% cream as four other commercial creams against ringworm and clinical isolates of four dermatophytes in vitro (51). Each of the commercial creams had clotrimazole, isoconazole nitrate, ketoconazole, benzoic acid, and salicylic acid as their main active ingredients.

It was found that essential oils were effective against both acute and chronic infections in humans. He also found that concentrations that were insufficient to kill the pathogenic organism in a laboratory were effective in humans. The example given was an in vitro minimum inhibitory concentration (MIC) of 0.00025 g/mL as opposed to an in vivo concentration of 0.0000032 g/mL(52).

Geranium, cinnamon, and peppermint were found by Viollon, et al., (53) to be effective in vitro against *Candida*. Citral is the generic name for two different isomeric aldehydes (geranial and neral) that are found in many essential oils. Citral is thought to be the component most likely to be antifungal (54) (Pattnaik et al., 1997). Onawunmi (55) found citral to have antifungal properties in dilutions as low as 0.005% to 0.008%. Essential oils containing large amounts of citral are melissa, verbena, and lemongrass. Aldehydes are best avoided on a damaged mucous membrane, but they can be used diluted on the skin. A component of essential oils found by Beylier and Givaudan (56) to have anti-candida properties is citronellol. Citronellol is an alcohol and is the main constituent of lemon grass and *Eucalyptus citriadora* (60%-80%). Alcohol is safe to use on the skin and the mucous membrane.Pattnaik et al.,(57) reported that lemongrass, *Eucalyptus globulus*, palmarosa, and peppermint were the most effective essential oils tested against cryptococcus. Basil and thyme were not included in this study. (Lemongrass was effective not only against cryptococcus but against all 11 other fungi tested in low dilutions.)

Table 1. Qualitative chemical analysis essential oils

Organisms	Chemicals tested					
	Alcohols	*Terpenoid*	*Phenolics*	*Flavonoids*	*Alkaloids*	*Aldehydes*
Cymbopogon citratus	+	+	+	+	+	+
Cymbopogon martinii	+	+	+	+	+	+
Cinnamomum zeylanicum	+	+	+	+	+	+
Rosmarinus officinalis	+	+	+	+	+	+
Mentha piperita	+	+	-	+	+	-
Pelargonium graveolens	+	+	-	-	+	-
Vitex negundo	-	+	+	+	+	-

(+) Present (-) Absent

Table 2. Antifungal activity of essential oils against clinical isolates from HIV positive individuals

Organisms	Zone of Inhibition							
	C.albicans	*C.tropicalis*	*C.neoformans*	*A.niger*	*A.flavus*	*A.fumigatus*	*Mucor spp*	*Rhizopus spp*
Cymbopogon citratus	9.87±0.73 ^{a}	11.08±0.38 ^{a}	13.0±0.51^{a}	11.07±0.35^{a}	8.13±0.636 ^{a}	12.09±0.62^{a}	14.25±0.97 ^{a}	10.01±0.60^{a}
Cymbopogon martinii	13.25±0.90^{b}	13.16±0.53^{b}	13.89±0.43^{a}	11.95±0.40^{b}	11.03±0.48^{b}	12.97±0.45^{a}	12.07±0.6 ^{1bc}	11.95±0.68^{b}
Cinnamomum zeylanicum	19.23±0.80^{c}	21.83±0.74^{c}	19.20±o.75^{b}	21.15±0.86^{b}	17.02±0.37^{c}	14.23±0.60^{b}	11.87±0.77^{ea}	18.43±1.47^{c}
Rosmarinus officinalis	13.12±0.54^{db}	9.72±0.70^{d}	12.96±0.41^{a}	11.12±0.66^{a}	7.97±0.73^{ah}	10.71±0.78^{c}	14.08±0.70^{ah}	10.07±0.61^{ag}
Mentha piperita	19.40±0.73^{ac}	12.14±0.48^{e}	11.13±0.64^{c}	13.18±0.77^{c}	10.68±0.76^{db}	8.11±0.37^{d}	11.12±0.57^{dbc}	213.06±0.67^{db}
Pelarogonium graveolens	13.13±0.46^{fb}	13.17±0.31^{fb}	9.95±0.50^{d}	9.03±0.58^{d}	12.0±0.48^{ebg}	13.12±0.37^{ea}	17.51±0.96^{e}	18.04±0.55^{e}
Vitex negundo	12.09±0.59^{gb}	8.99±0.47^{gd}	10.09±0.42^{ed}	13.03±0.81^{eac}	11.08±0.71^{fb}	9.18±0.57^{f}	12.06±0.58^{fbc}	13.09±0.43^{fb}
Miconazole ©	14.15±0.71^{hb}	12.12±0.62^{he}	11.01±0.81^{fce}	13.51±0.51^{fdec}	12.09±0.61^{gf}	14.18±0.77^{gb}	12.11±0.56^{gbc}	10.98±0.46^{abh}

© - control antibiotic disc in 100 µg concentration

Different superscripts in the same column are significantly different at $P<0.05$ level (Least Significance Difference) mean followed by ± S.D.

Table 3. MICs of essential oils against clinical fungal isolates from HIV positive individuals

Organisms	MIC (µg/ml)							
	Cymbopogon citratus	*Cymbopogon martini*	*Cinnamomum zeylanicum*	*Rosmarinus officinalis*	*Mentha piperita*	*Pelarogonium graveolens*	*Vitex negundo*	Miconazole©
Candida albicans	250	250	15.6	125	1000	500	500	15.6
Candida tropicalis	250	250	15.6	125	1000	500	500	15.6
Cryptococcus neoformans	250	250	15.6	250	1000	500	500	15.6.
Aspergillus niger	500	500	31.25	500	1000	500	500	31.25
Aspergillus flavus	500	500	31.25	500	1000	500	500	31.25
Aspergillus fumigatus	500	500	31.25	500	1000	500	500	31.25
Mucor spp	500	500	31.25	500	1000	500	500	62.5
Rhizopus spp	500	500	31.25	500	1000	500	500	62.5

The MIC for each of the four essential oils against cryptococcus was 5 L/mL. In another article, Pattnaik, et al.,(58) found that complete essential oils were more effective against cryptococcus than the isolated, active component. There was one exception, lemongrass, which was equal to the isolated parts of citral and geranial. Larrondo and Calvo (59) compared the topical and inhaled action of citral to the systemic effects of clotrimazole. Although the actual way essential oils work as fungicides is not completely clear, it appears that metabolism and growth of the fungus are inhibited, often with a breakdown in the lipid part of the membrane, resulting in increased permeability and/or rupture (60).

Soliman *et al*., (61) tested essential oil of rosemary. They investigated the essential oil distilled from two plants growing in different climatic conditions. They found that both rosemary essential oils were effective against *C. neoformans* in vitro and recommended that either essential oil could be an effective treatment in AIDS patients with cryptococcal meningitis and pneumonia. Although both types of rosemary were effective, the effectiveness could have been due to a different chemical component in each oil. Many of the essential oils used showed good fungistatic action. The best effects were from palmarosa, geranium, savory, sandalwood, thyme, marjoram, and

Fig 1. Antifungal activity of essential oils against clinical isolates from HIV positive individuals

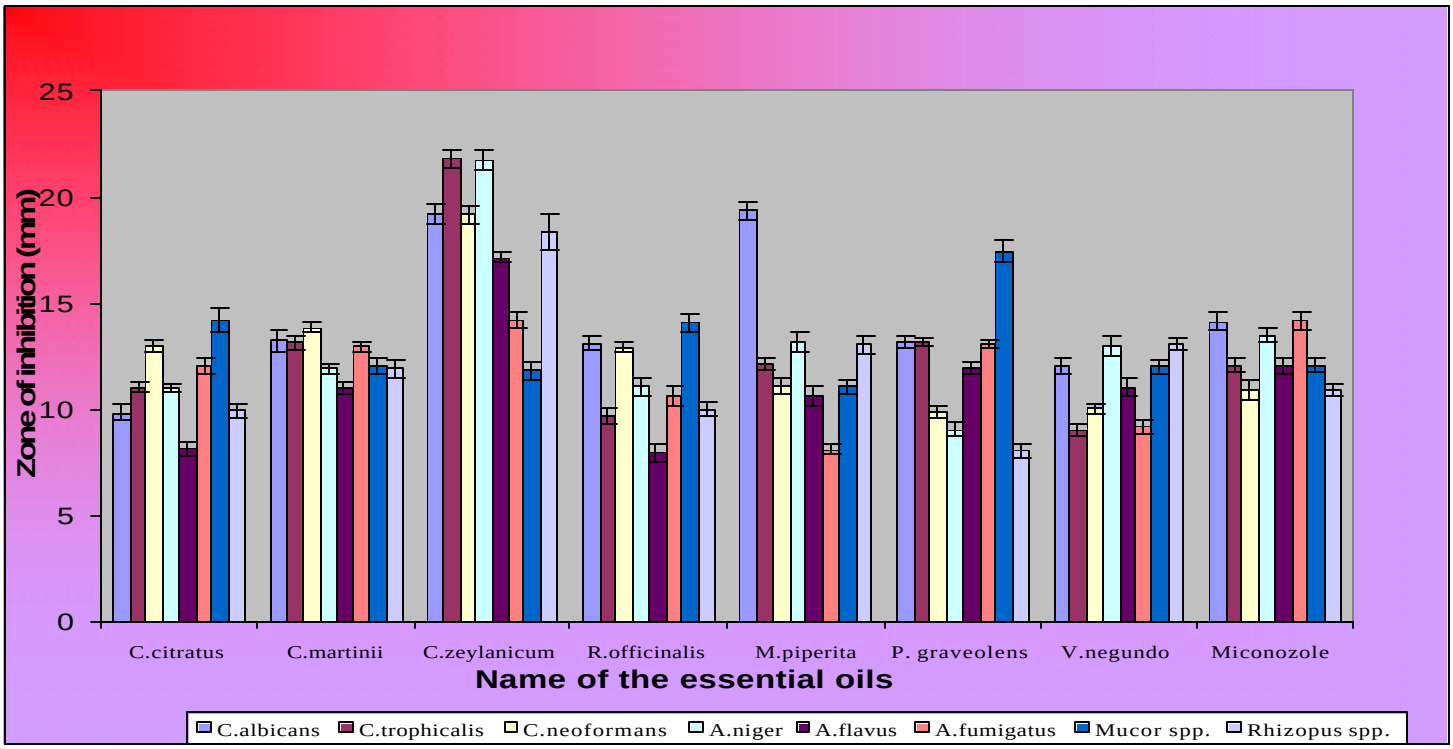
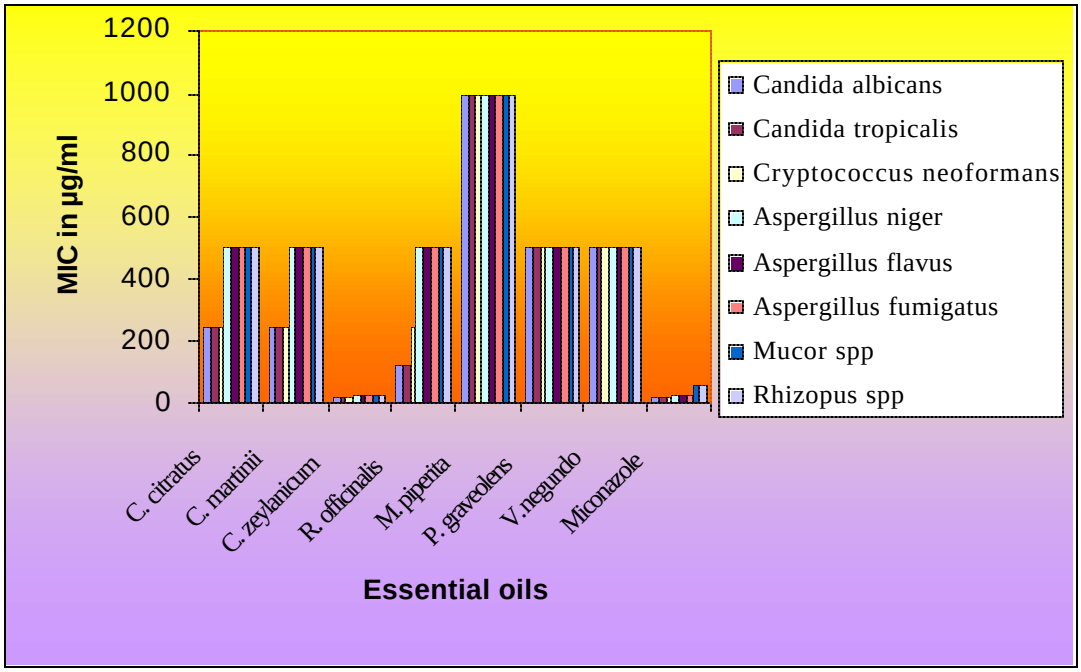


Fig. 2. MICs of essential oils against clinical fungal isolates from HIV positive individuals



lavender that appeared to agree with the findings of Valnet(62) as discussed earlier in this article and could be related to the adaptogenic capacity of essential oils (and all plant medicines) to behave differently depending on the terrain they are in.

Lucini, *et al*.,(63) indicated that mycelial growth inhibition is caused by the monoterpenes present in essential oils. These components would increase the concentration of lipidic peroxides such as hydroxyl, alkoxyl and alkoperoxyl radicals and so bring about cell death. For Sharma and Tripathi (64), the EOs would act on the hyphae of the mycelium, provoking exit of components from the cytoplasm, the loss of rigidity and integrity of the hypha cell wall,

resulting in its collapse and death of the mycelium.

Only few substances are known to inhibit human pathogenic fungi, which are often completely resistant to antibiotics, and most of them are relatively toxic. The increased incidence of therapeutic failure in the treatment of fungal infections and the prevalence of opportunistic infections has renewed interest in the search for new antifungal agents, including those obtained from higher plants. Present results allow supposing that these natural compounds could be useful agents in the topical treatment of fungal infections. Medicinal plants have been used in developing countries as alternative treatments to health problems. Many plant extracts and essential oils isolated from plants have been shown to exert biological activity in vitro and in vivo, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants (65). Brazil, Cuba, India, Jordan and Mexico are examples of countries that have a diverse flora and a rich tradition in the use of medicinal plants for both antibacterial and antifungical applications (66)(67)(68).

Cryptococcus neoformans, a fungus which causes infection during the last stages of AIDS is inhibited both by palmarosa oil and geraniol (96).

Potassium leakage from a different fungus, *C. albicans*, due to action of geraniol over a period of 2 h has been reported earlier (70). Palmarosa oil led to changes in the composition of the yeast cell membrane, with more saturated and less unsaturated fatty acids in the membrane after exposure of *S. cerevisiae* cells to the oil. Some of the palmarosa oil was lost by volatilization during incubation of the oil with the yeast cells. The actual concentration of the oil components affecting the yeast cells could not therefore be accurately determined (71).

Reports of some essential oils affecting membrane integrity inc!ude tea tree oil causing damage to membranes in *C. albicans* while other oils and their components have disrupted the permeability barrier of yeast cells (72). The fatty acid composition of microbial cell membranes affects their ability to survive in various environments (73). The ratio of saturated to unsaturated fatty acids can alter in response to environmental conditions (74). Maintenance of an optimal degree of fluidity of membrane lipids is important for normal function, with environmental adaptation known as homeoviscosic adaptation(75)(76).

Conclusions

The essential oils as antimicrobial agents present two main characters: the first is their natural origin which means more safety to the people and the environment, the second is that they have been considered at low risk for resistance development by pathogenic microorganisms. The antifungal activity is attributed to the components of essential oils, which mostly interferes with the cell membrane structure and function According to the results of this study, the essential oil or their ethanol extract of those essential oils may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of fungal diseases in various patients, particularly, for HIV/AIDS patients.

REFERENCES

1. Walensky RP, Paltiel AD, Losina E, et al. The survival benefits of AIDS treatment in the United States. J Infect Dis 2006;194:11–9.
2. Gebo KA, Fleishman JA, Reilly ED, Moore RD. High rates of primary Mycobacterium avium complex and Pneumocystis jiroveci prophylaxis in the United States. Med Care 2005;43(Suppl. 9):23–30.
3. Bonnet F, Lewden C, May T, et al. Opportunistic infections as causes of death in HIV-infected patients in the HAART era in France. Scand J Infect Dis 2005;37:482–7.
4. Teshale EH, Hanson DL, Wolfe MI, et al. Reasons for lack of appropriate receipt of primary Pneumocystis jiroveci pneumonia prophylaxis among HIV-infected persons receiving treatment in the United States: 1994–2003. Clin Infect Dis 2007;44:879–83.
5. Gebo KA, Fleishman JA, Moore RD. Hospitalizations for metabolic conditions, opportunistic infections, and injection drug use among HIV patients: trends between 1996 and 2000 in 12 states. J Acquir Immune Defic Syndr 2005;40:609–16.
6. Betz ME, Gebo KA, Barber E et al. Patterns of diagnoses in hospital admissions in a multistate cohort of HIV-positive adults in 2001. Med Care 2005;43:1113–4.
7. Buchecz K, Baker R, Moorman AC, et al. Rates of hospitalizations and associated diagnoses in a large multistate cohort of HIV patients in the United States, 1994-2005. AIDS 2008;22(11):1345-54.
8. Louie JK, Hsu LC, Osmond DH, Katz MH, Schwarcz SK. Trends in causes of death among persons with acquired immunodeficiency syndrome in the era of highly active antiretroviral therapy, San Francisco, 1994–1998. J Infect Dis 2002;186:1023–7.
9. Palella FJ, Jr., Baker RK, Moorman AC, et al. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 2006;43:27–34.108 MMWR April 10, 2009
10. Smit C, Geskus R, Walker S. et al. Effective therapy has altered the spectrum of cause-specific mortality following HIV seroconversion. AIDS 2006;20:741–9.
11. Reichling J. Plant-microbe interaction and secondary metabolites with antiviral, antibacterial and antifungal properties. In: Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology, Ann Plant Rev —Wink M, ed. (1999;) 3:. Sheffield: Sheffield Academic Press. 187–273.
12. Golab M, Burdzenia O, Majewski P, Skwarlo-Sonta K. Tea tree oil inhalations modify immunity in mice. J Appl Biomed (2005;) 3:: 101–8.
13. Naser B, Bodinet C, Tegtmeier M, Lindequist U. Thuja occidentalis (Arbor vitae): A review of its pharmaceutical, pharmacological and clinical properties. eCAM (2005;) 2:: 69–78.
14.Ito N, Nagai T, Oikawa T, Yamada H, Hanawa T. Antidepressant-like effect of l-perillaldehyde in stress-induced depression-like model mice through regulation of the olfactory nervous system. In: eCAM (2008;).
15. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol (1998 ;) 62:: 183–93.
16.Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA. Antibacterial activity of Quercus ilex bark's extracts. J Ethnopharmacol (2007;) 112:: 426–9.
17.Salomao K, Pereira PR, Campos LC, Borba CM, Cabello PH, Marcucci MC, de Castro SL. Brazilian Propolis: correlation between chemical composition and antimicrobial activity. eCAM (2008;) 5:: 317–24.
18. Mitscher, L.A., S. Drake, S.R. Gollapudi and S.K. Okwute, 1987. A modern look at folkloric use of anti-infective agents. J. Natural Products, 50: 1025-1040.
19. Morris, J.A., A. Khettry and E.W. Seitz, 1979. Antimicrobial activity of aroma chemicals and essential oils. J. Am. Oil Chemists' Soc., 56: 595-603.
20. Ross, S.A., N.E. El-Keltawi and S.E. Megalla, 1980. Antimicrobial activity of some Egyptian aromatic plants. Fitoterapia, 51: 201-205.
21. Yousef, R.T. and G.G. Tawil, 1980. Antimicrobial activity of volatile oils. Die Pharmazie. 35: 698-701.
22. Hili, P., C.S. Evans and R.G. Veness, 1997.Antimicrobial action of essential oils: the effect of dimethylsulfoxide on the activity of cinnamon oil. Letters in Appl. Microbiol., 24: 269-275.
23. Lis-Balchin, M. and S.G. Deans, 1997. Bioactivity of selected plant essential oils against Listeria monocytogenes. J. Appl. Bacteriol., 82: 759-762.
24. Aggarwal, K.K., A. Ahmad, T.R. Santha, N. Jain, V.K. Gupta, K. Sushil and S.P. Khanuja, 2000. Antimicrobial activity spectra of *Pelargonium graveolens* L. and *Cymbopogon winterianus* Jowitt. oil constituents and acyl derivatives. J. Med.Aromatic Plant Sci., 22: 544-548.
25. Singh, H.B., 2000. Antifungal efficiency of some essential oils against *Sclerotium r*44: 71-73.*olfsii*. Ind. Perf.
26. Quale, J.M., D. Landman, M.Z. Zaman, S. Burney anS. Sathe, 1996. *In vitro* activity of *Cinnamomum*d *zeylanicum* againfor oral Candidiasis. Am. J. Chinese Med.,st azole resistant and sensitive*Candida* Species and a pilot study of Cinnamon24: 103-109.
27. Viollon, C. and J.P. Chaumont, 1994. Antifungal properties of essential oils and their components upon *Cryptococcus neoformens*.ain Mycopathologia. 128: 151-153
28. Chaumont, J.P., 2003. Antimycotic essential oils: Impact on skin micro flora, in Plant-DerivedAntimycotics: Current Trends and Future Prospects (Eds M.K. Rai and D. Mares). Haworth press, USA, 357-364.
29. Matan, N., H. Rimkeeree, A.J. Mawson, P. Chompreeda, V. Haruthaith2006. Antimicrobial activity of cinnamon and cloveanasan and M. Parker, oils under modified atmosphere conditions. Intl. J. Food Microbiol., 107(2): 180-5.
30. Kishore, N., A.K. Mishra and J.P. Chansouria, 1993. Fungal toxicity of essential oils againsdermatophytes. Mycoses. 36: 211-225.t
31. J.J. Kabara, Phenols and chelators. In: N.J. Russell and G.W. Gould, Editors, *Food preservatives*, Blackie, Glasgow (1991), pp. 200–214.
32. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen and M. Polissiou, Antimicrobial and antioxidant activities of the essential oils and various extracts of *Salvia tomentosa* Miller (Lamiaceae), *Food*

Chemistry **90** (2005), pp. 333–340.
33. Trease GE, Evans WC (1989) Textbook of Pharmacognosy. 12th Edn. Balliere, Tinadl London.
34. Harborne JB (1998). Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London. pp. 182-190.
35. Sofowora A (1984). Medicinal plants and traditional medicine in Africa published in association with spectrum Books Ltd. Ibadan by John Wiley and Sons, NY pp 142-143.
36. Harborne JB (1993).Phytochemical method. 3rd Ed. Chapman and Hall, London. pp. 135-203.
37. Perez, C., Agnese, A.M., Cabrera, J.L., (1999). The essential oil of *Senecio graveolens* (Compositae): chemical composition and antimicrobial activity tests. *J. Ethnopharmacol*.66,91–96.
38. Bagamboula, C.F., Uyttendaele, M., Debevere, J., (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiol*. 21, 33–42.
39. Erdemog¡lu, N., Ku¨ peli, E., Yes_ilada, E., (2003). Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. *J.Ethnopharmacol*. 89, 123–129.
40. Perez, C., Pauli, M., Bazerque, P., (1990). An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp*. 15, 13– 115.
41. Alves, S.H., Cury, A., 1992. Estudo comparative entre as te´cnicas de diluic¸a˜o em caldo para *Candida*. Revista de Patologia Tropical 34, 259–262.
42. Sowell, R., Moneyham, L.,&Arnada-Naranjo, B. (1999). The care of women with AIDS. *Nursing Clinics of North America, 34*, 179-198.
43. Beal, M.,&Nield-Anderson, L. (2000). Acupuncture for symptom relief in HIV positive adults: Lessons learned from a pilot study. *Alternative Therapies in Health and Medicine*, *6*(5), 33-42.
44. Dwyer, J. T., Salvato-Schille, A. M., & Couston, A. (1995). The use of unconventional remedies among HIV positive men living in California. *Journal of the Association of Nurses in AIDS Care*, *6*(1), 17-28.
45. Elion, R., & Cohen, C. (1997). Complementary medicine and HIV infection. *Complementaryand Alternative Therapies in PrimaryCare*, *24*, 905-919.
46. Targ, E. (2000). CAM and HIV/AIDS: The importance of complementarity. *Alternative Therapies in Health and Medicine*, *6*(5), 30-31.
47. Buckle, J. (1999b).Use of aromatherapy as a complementary treatment for chronic pain. *Alternative Therapies in Health and Medicine*, *5*(5), 42-55.
48. Styles, J. (1997). The use of aromatherapy in hospitalized children with HIV disease. *Complementary Therapies in Nursing and Midwifery*, *3*, 16-21
49. Buckle, J. (2000). Lavender for chronic pain. *Holistic Nursing Update*, *1*(5), 36-39.
50. Tisserand, R., & Balacs, T. (1995). *Essential oil safety*. London: Churchill Livingstone.
51. Wannissorn, B., Jarikasen, S., & Soontorntanasart, T. (1996).Antifungal activity of lemongrass oils and lemongrass oil cream. *Physiotherapy Research*, *10*, 551-554.
52. Valnet, J., Duraffourd, C., Duraffourd, P.,&Lapraz, J. (1978).New results and interpretations of 268 clinical tests using an aromatogram. *Plantes Medicinales et Phytotherapie*, *12*(10), 43-52.
53. Viollon, C., Leger, D., & Chaumont, J. (1993). The antagonistic properties in vitro, of specified natural volatile compounds with respect to the germs of the vaginal flora. *Plantes Medicinales et Phytotherapie*, *26*(1), 17-22.
54. Pattnaik, S., Subramanyam, V., Bapaji, M., & Kole, C. (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios*, *89*, 39-46.
55. Onawunmi, G. (1989). Evaluation of the antimicrobial activity of citral. *Letters of Applied Microbiology*, *9*, 105-108.
56. Beylier, M.,&Givaudan, S. (1979). Bacteriostatic activity of some Australian essential oils. *Perfume and Flavorist, 4*, 23-25.
57. Pattnaik, S., Subramanyam, V.R., Kole, C., 1996. Antibacterial and antifungal activity often essential oils in vitro. Microbios 86, 237– 246.
58. Pattnaik, S., Subramanyam, V.R., Bapaji, M., Kole, C.R., 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89, 39–46.
59. Larrondo, J., & Calvo, M. (1991). Effect of essential oils on *Candida albicans*: A scanning electron microscope study. *Biomedical Letters*, *46*, 269-272.
60. Larrondo, J., Agut, M., & Calvo-Torres, M. (1995). Antimicrobial activity of essences from labiates. *Microbios*, *82*, 171-172.
61. Soliman, F., El-Kashoury, E., Fathy, M., & Gonaid, M. (1994). Analysis and biological activity of the essential oil of *Rosmarinus officinalis L* from Egypt. *Flavour and Fragrance Journal*, 9,29-33.
62. Valnet, J., Duraffourd, C., Duraffourd, P.,&Lapraz, J. (1978).New results and interpretations of 268 clinical tests using an aromatogram. *Plantes Medicinales et Phytotherapie, 12*(10), 43-52
63. Lucini et al., 2006 E.I. Lucini, M.P. Zunino, M.L. López and J.A. Zygadlo, Effect of monoterpenes on lipid composition and sclerotial development of *Sclerotium cepivorum* Berk, *Journal of Phytopathology* **154** (2006), pp. 441–446.
64. Sharma, N., & Tripathi, A. (2006b). Effects of *Citrus sinensis* (L.) Osbeck epecarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*, Available on line.
65. Mart´ýnez, M.J., Betancourt, J., Alonso-Gonz´alez, N., Jauregui, A., 1996.Screening of some Cuban medicinal plants for antimicrobial activity. Journal of Ethnopharmacology 52, 171–174.
66. Mahasneh, A.M.A., Adel, M.A., El-Oqlah, A.A.B., 1999. Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. Journal of Ethnopharmacology 64, 271–276.
67. Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74, 113–123.
68. Rehder, V.L.G., Machado, A.L.M., Delarmelina, C., Sartoratto, A., Duarte, M.C.T., Figueira, G.M., 2004. Composic¸ ˜ao qu´ýmica e atividade antimicrobiana do ´oleo essencial de duas esp´ecies de Origanum. Revista Brasileira de Plantas Medicinais 6, 67–71.
69. Viollon, C. and J.P. Chaumont, 1994. Antifungal properties of essential oils and their components upon *Cryptococcus neoformens*.ain Mycopathologia. 128: 151-153
70. Bard, M., Albrecht, M.R., Gupta, N., Guynn, C.J., Stiliwell, W., 1988. Geraniol interferes with membrane functions in strains of *Candida* and *Saccharomyces*. Lipids 23, 534–538.
71. Anjali Prashar, Pauline Hili, Robert G. Veness and Christine S. Evans, 2003. Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae* Phytochemistry, Volume 63, Issue 5, , Pages 569-575.
72. Cox, S.D., Gustafson, J.E., Mann, C.M., Markham, J.L., Liew, Y.C., Hartland, R.P., Bell, H.C., Warmington, J.R., Wyllie, S.G., 1998. Tea tree oil causes K+ leakage and inhibits respiration in Escherichia coli. Lett. Appl. Microbiol. 26, 355–358.
73. Ghfir, B., L, F.J., Koulali, Y., Ecalle, R., Dargent, R., 1994. Effect of essential oil of Hyssopus officinalis on the lipid composition of *Aspergillus fumigatus*. Mycopathologia 126, 163–167.
74. Odumeru, J.A., Damore, T., Russel, I., Stewart, G.G., 1993. Alterations in fatty acid composition and trehalose concentration of saccharomyces brewing strains in response to heat and ethanol shock. J. Ind. Microbiol 11, 113–119.
75. Heipieper, H.J., de Bont, J.A., 1994. Adaptation of *Pseudomonas putida* S12 to ethanol and toluene at the level of fatty acid composition of membranes. Appl. Environ. Microbiol. 60, 4440–4444.
76. Rotert, K.R., Toste, A.P., Steiert, J.G., 1993. Membrane fatty acid analysis of Antarctic bacteria. FEMS Microbiol. Lett. 114, 253–257.

Source of support: Nil, Conflict of interest: None Declared