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RESEARCH ARTICLE

Analysis On Chemical Composition Of Natural And Synthetic Essentials Oils Of*pelargonium Graveolens* (Geranium)By Gc-Ms And Their Antimicrobial Activity Against Human Pathogenic Bacteria And Fungi

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ABSTRACT

The natural and synthetic essential oil from the plant *Pelargonium graveolens* were selected for anti bacterial and antifungal activity on selected bacterial and fungal pathogens. The natural and synthetic oil was preparedseparately with Dimethyl sulfoxide (DMSO)at 25µl, 50µl and 75µl concentration for the sensitivity test. The antimicrobial activity of plant essential oil was tested against clinical isolates. The results showed that natural *Pelargonium graveolens* oil showed very high effective activity than the synthetic oil at all the three different concentrations. GC/MSanalysis was done to know the chemical comparison of *Pelargonium graveolens* natural and synthetic essential oils. The chemical compound geraniol present in *Pelargonium graveolens* natural oil may be showed high activity against the bacterial and fungal isolates.

INTRODUCTION

Essential oils are volatile, natural, complex compounds that are produced by plants as secondary metabolites for protection against bacteria, viruses, fungi and pests (Rota *et al*., 2008). They also have an important role in dispersion of pollens and seeds by attracting some insects.

In Middle Ages essential oils were used for preservation of foods and as flavoring antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies (Baydar*et al*., 2004;Demirci*et al*., 2008;Sagdi and ozcan, 2003). But characterizing these properties in laboratory dated back to the early 1900s.

At present, about 3000 essential oils are known and 300 of them are used commercially in different industries such as pharmaceutical, agronomic, food, sanitary, cosmetic and perfume (Bakkali*et al*., 2008). Today, antioxidant, antitumor and antiviral, antifungal and antibacterial activity of *P.graveolensessential* oils and their constituents is widely studied (Lee *et al*., 2012;Shanab *et al*., 2011;Zanetti *etal*., 2010;Crespo-ortiz and wei, 2012;Campelo *et al*., 2011;Astani *et al*., 2010;Vukovic *et al*., 2007;Zeng *et al*.,2011;Furletti *et al*., 2011). In the other hand, Antimicrobial resistance (AMR) is now a global concern.

The *Pelargonium* (*Geraniaceae*) genus is represented by many essential oil producing species*inter alia*: *P. graveo lens*, *P. odoranissimum*, *P. zonale*and and*P. roseum*. Geranium oil is obtained from leaves, flowers and stalks by steam or hydro distillation. The therapeutic effects of the oil find application in the treatment of dysentery, diarrhoea, biliary conditions, gastric ulcers, diabetes, cancer and skin diseases. The main constituents responsible for biological activity are citronellol, geraniol, linalool, isomenthone, nerol and citronellylformate (Lis-Balchin and Geranium,2004;Verma*et al*., 2011;Shawl *et al*., 2006). Due to these components the essential oil from *P. graveolens* has a strong antibacterial and antifungal effect.

The overuse of antimicrobial chemotherapeutic agents, unfortunately typical of modern medicine, isevident and cannot be glossed over in silence.

Thus the search for effective and safe medicines thatcould be used to treat staphylococcal infections is on. We have decided to determine if the essential oil derived from *Pelargonium graveolens* and it has antimicrobial properties against clinical *S. aureus* and *A.flavus*isolates, what could make it an alternative or complementary toantibiotic therapy.

MATERIALS AND METHODS

Essential oils

The natural and synthetic essential oil geranium was purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India). GC-MS technique was done to know the composition of *Pelargoniumgraveolens* natural and synthetic geranium essential oils.

Chemicals and microorganisms

All chemicals with the highest purity available and culture media were purchased from Himedia Mumbai, Maharashtra (India). *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis*, *Candida kefyr.Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*(Clinical isolates) was used as test organisms.

The bacteria and fungi were obtained from Microlabs Institute of Research and Technology Arcot, Tamil Nadu (India).

GC/MS analysis of *Pelargonium graveolens*, natural and synthetic essential oils

GC-MS was done at South Indian Textile Research

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Association Coimbatore, Tamil Nadu (India) and analysis was carried out using a Hewlett-Packard 5890/5971A system fitted with a HP1 column (50 m x 0.20 mm fused silica capillary column; film thickness, 0.5 µm). GC oven initial temperature was 60°C and was programmed to 220°C at a rate of 2°C/min and 220°C during 120 min under the following operation conditions: vector gas, He; injector and detector temperatures, 250°C; injected volume: 0.2 µl, with a ratio split of 1/100. Retention indices were determined with Hexane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35 - 400 amu. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial (Wiley, MassFinder 2.1 Library, NIST98) and home-made libraries and MS literature data (McLafferty and Stauffer, 1989; Adams, 1995; Joulain and König, 1998; Joulain*et al.*, 2001)

Determination of antimicrobial activity

In this study standard agar well diffusion methodwas followed (Perez *et al*., 1990; Perez *et al*., 1999;Erdemogllu*et al*., 2003; Bagamboula*et al*., 2004).The fungal isolate was suspended in Potato Dextrose broth and the bacterial isolate in nutrient broth Himedia Mumbai, Maharashtra(India) broth and diluted to approximately 10^5colony forming unit (CFU) per mL. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 15µl of the samples solutions were delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Dime thy l sulfa oxide was used as solvent control. Amphotericin B was used as reference antifungal agent for molds, fluconazole was for yeast like fungi and ciprofloxazin for bacteria. The tests were carried out in triplicate.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P< 0.05$ using Duncan's multiple range test (by SPSS software)Version 9.1

Table 1 Chemical composition of natural *Pelargonium graveolens* oil

Compound Name	Retention time(min)	Area %
Geraniol	8.87	0.36
Lavanduyl acetate	17.86	0.41
Farnesol	13.39	0.34
6-Octen-1-ol, 3,7-dimethyl-, acetate	9.95	3.79
Citronellyl propionate	9.95	3.79
Cis- farnesol	13.99	0.36
Geranyl acetate	9.31	6.54
Trans-geraniol	13.39	0.41
Geraniol formate (CAS)	6.54	9.31
Neryl acetate	5.95	3.45

RESULTS

Chemical composition of natural and synthetic *Pelargonium graveolens*essential oils from GC-MS

Chemical compositions of natural and synthetic *Pelargonium graveolens*essential oils are shown in (Tables 1and 2). As seen, major components of natural and synthetic *Pelargonium graveolens* essential oilswere: Geraniol (0.36%), Geraniol formate (9.31%), farnesol (0.34%), trans-Geraniol (0.41%),Citronellyl acetate (3.79%),Geranyl acetate (6.54%) respectively.

Table 2 Chemical composition of synthetic *Pelargonium graveolens* oil

Compound Name	Retention time(min)	Area %
Geranyl acetate	9.21	6.94
Lavanduyl acetate	11.33	0.73
Geranyl tiglate	16.69	3.96
Farnesyl acetate 3	19.02	0.38
Benzyl benzoate	18.08	5.53
Geranyl vinyl ether	21.93	0.19
2,4-Octadienoic acid,	23.68	0.17
Citronellyl acetate	24.89	0.26
Guaiol	16.14	1.76
Neryl acetate	10.31	1.40

***In vitro* antimicrobial activity**

The natural and synthetic geranium oil showed effective antimicrobial activity against the bacterial and fungal isolates.In agar well diffusion method the selected essential oils were effective against all forms such as yeast like fungi and molds and bacteria.(Table 3).

The *Pelargonium graveolens*natural oil 75µl mixed with 25µl of DMSO showed high activity against *Escherichia coli*(13.06±0.40) least against *Bacillus subtilis*(4.00±0.10)and in *Pelargonium graveolens* synthetic oil75µl mixed with DMSO 25µl at showed higher activity in *Klebsiella Pneumoniae* (7.06±0.30) and low activity against *Staphylococcus aureus* (3.00±0.00).(Table 4)The *Pelargonium graveolens* natural oil 50µl mixed with Dmso at 50µl showed high activity against *Staphylococcus aureus* (12.50±0.50) least against *Bacillus subtilis*(9.00±0.30)and in *Pelargonium graveolens* synthetic oil 50µl mixed with Dmso 50µl at showed higher activity in *Klebsiella Pneumoniae* (8.00±0.30) and low activity against *Bacillus cereus* (3.96±0.05).(Table 5) The geranium natural oil 25µl mixed with DMSO at 75µl showed high activity against *Bacillus cereus* (11.00±0.50) least against *Bacillus subtilis*(4.00±0.00)and in *Pelargonium graveolens* synthetic oil 25µl mixed with DMSO 75µl at showed higher activity in *Klebsiella Pneumoniae* (7.03±0.25) and low activity against *Escherichia coli* (4.00±0.00)(Table 6)The *Pelargonium graveolens* natural oil 75µl mixed with Dmso at 25µl showed high activity against *Aspergillus niger* (18.00±0.50) least against *Aspergillus flavus*(4.00±0.00)and in *Pelargonium graveolens* synthetic oil 75µl mixed with Dmso 25µlshowed higher activity in *Aspergillus niger* (10.96±0.35) and low activity against *Candida kefyr* (6.00±0.20).(Table 7)The geranium natural oil 50µl mixed with DMSO at 50µl showed high activity against *Candida kefyr* (11.00±0.36) least against *Candida albicans* (6.00±0.10)and in geranium synthetic oil 50µl mixed with DMSO 50µl at showed higher activity in *Aspergillus niger* (11.10±0.36) and low activity against *Candida tropicalis* (4.00±0.00).(Table 8)The geranium natural oil 25µl mixed with DMSO at 75µl showed high activity against *Aspergillus flavus* (14.96±0.45) least against *Candida kefyr* (5.03±0.15)and in geranium synthetic oil 25µl mixed with Dmso 75µl at showed higher activity in *Aspergillus flavus* (10.96±0.45) and low activity against *Candida kefyr* (3.00±0.00).

All bacteria and fungi were found to be sensitive to all test essential oils and mostly comparable to the standard reference antifungal drug Amphotericin B, fluconazole and bacteria for ciprofloxacin to higher extent.

Table 3 Antibacterial activity of Pelargonium *graveolens* natural and synthetic oil

Microorganisms	Geranium naturaloil	Geranium synthetic oil	Geranium natural Oil 75 µl &DMSO25 µl	Geranium synthetic Oil 75 µl &DMSO25µl	Ciprofloxacin
Staphylococcus aureus	8.96±0.45^{a}	4.93±0.11^{a}	5.10±0.17^{a}	3.00±0.00^{a}	20.50±0.50^{a}
Klebsiella pneumoniae	12.96±0.45^{b}	8.10±0.36^{b}	11.96±0.40^{b}	7.06±0.30^{b}	20.00±1.00^{a}
Escherichia coli	14.00±0.50^{c}	6.03±0.15^{c}	13.06±0.40^{c}	4.93±0.11^{c}	21.33±0.57^{b}
Bacillus cereus	10.06±0.30^{d}	5.80±0.26^{d}	11.06±0.30^{b}	4.10±0.17^{c}	24.00±1.00^{c}
Bacillus subtilis	8.03±0.25^{a}	4.93±0.11^{a}	4.00±0.10^{d}	3.00±0.00^{a}	22.83±0.76^{d}

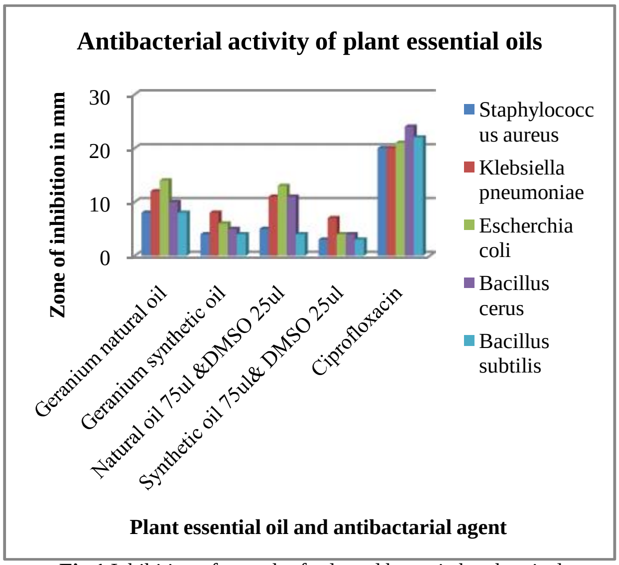

Fig 1 Inhibition of growth of selected bacteria by chemical compoundsof two essentialoils

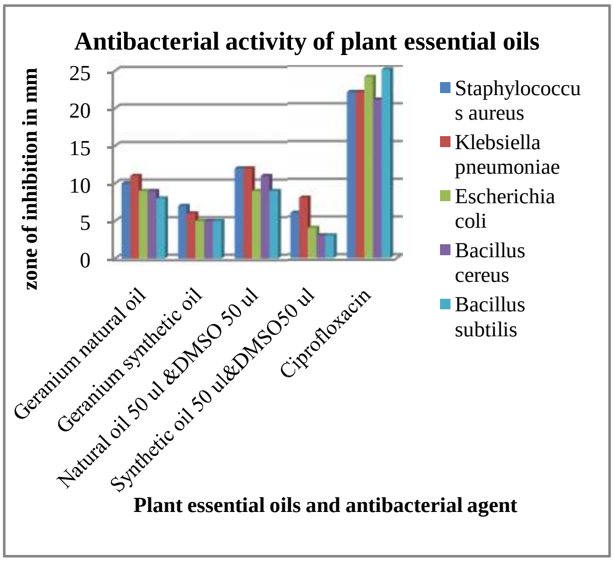

Fig 2 Inhibition of growth of selected bacteria by chemical compoundsof two essentialoils

Table 4

Microorganisms	Geranium natural oil	Geranium synthetic oil	Geranium natural Oil 50 µl &DMSO50 µl	Geranium synthetic Oil 50 µl &DMSO50 µl	Ciprofloxacin
Staphylococcus aureus	10.00±0.50^{a}	7.00±0.30^{a}	12.50±0.50^{a}	6.00 ±0.00^{a}	22.00±1.00^{a}
Klebsiella pneumoniae	11.00±0.50^{b}	6.03±0.25^{b}	12.00±0.50^{a}	8.00±0.30^{b}	22.00±1.00^{a}
Escherichia coli	9.96±0.45^{c}	5.00±0.00^{c}	9.03±0.25^{b}	4.00±0.00^{c}	24.66±1.15^{b}
Bacillus cereus	9.03±0.25^{c}	5.03±0.15^{c}	11.50±0.50^{c}	3.96±0.05^{d}	21.00±1.0(
Bacillus subtilis	8.96±0.45^{d}	5.03±0.15^{c}	9.00±0.30^{b}	3.96±0.15^{d}	25.00±1.00^{d}

The values are represented as the Mean ± SD of two essential oils. These essential oils have significant effect at 0.05 levels

Table 5

Microorganisms	Geranium naturaloil	Geranium synthetic oil	Geranium natural Oil 25 µl &DMSO75µl	Geranium synthetic Oil 25 µl &DMSO75µl	Ciprofloxacin
Staphylococcus aureus	3.96±0.05^{a}	5.03±0.25^{a}	9.03±0.25^{a}	7.00±0.20^{a}	29.00±1.00^{a}
Klebsiella pneumoniae	7.06±0.30^{b}	4.00±0.00^{b}	9.00±0.30^{a}	7.03±0.25^{a}	30.00±1.00^{b}
Escherichia coli	11.00±0.50^{c}	5.03±0.15^{c}	10.00±0.50^{b}	4.00±0.00^{b}	30.00±1.00^{b}
Bacillus cereus	9.03±0.25^{d}	5.06±0.11^{c}	11.00±0.50^{c}	5.00±0.10^{c}	32.00±2.00^{c}
Bacillus subtilis	11.10±0.36^{c}	5.03±0.25^{c}	4.00±0.00^{d}	6.06±0.20^{d}	28.00±1.00^{d}

The values are represented as the Mean ± SD of two plant essential oils. These essential oils have significant effect at 0.05 levels

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 against bacterial and fungal pathogens.

The obtained results, in accordance with the literature, show that geranium oil has antimicrobial properties against all tested strains.

The activity is due to the high content of alcoholic compounds with antibacterial properties such as citronellol and geraniol, which account for over 40% of the ingredients of the geranium oil (Lis-balchin and Deans, 2007;Fabio*et al*., 2007).

The plant essential oil *Pelargonium graveolens* was also sensitive to the control antibiotics (Ciprofloxazin and Amphoterecin -B). The essential oils of the plant were active against all of the bacteria and fungi the susceptibility of the strains changed with the dilution of essential oils in DMSO. The pure and neat essential oils showed the most extensive inhibition zones and they were very effective antimicrobial system along with ciprofloxazin and amphotericin-B. The activity of the oils would be expected to relate to the composition of the plant essential oils and possible synergistic interaction between components. High proportions of *β*-citronellol and caryophyllene oxide in our oils make them interesting and valuable subjects for food, medicine, aromatherapy and cosmetics industries where an antiseptic, clean and fresh characteristics flavor and fragrance is desired. The food protective and antimicrobial properties of *β*-citronellol as prominent part of *P. graveolens* volatile oil

(Jeon*et al*., 2009) and antifungal, analgesic and anti-inflammatory activities of caryophyllene oxide as famous for volatile oil (Chavan*et al*., 2010;Yang *et al*.,1999) can be considered and may be suitable for use in further nutraceutical and pharmaceutical vehicles.

antiseptic preparations, and be used in the hospitals, nursing homes and clinics. The application of essential oils in the treatment of human diseases, particularly infectious diseases caused by multidrug resistant bacterial strains, may be an interesting alternative to synthetic drugs.

Table 6 Antifungal activity of *Pelargonium graveolens* natural and synthetic oil

Microorganisms	Geranium naturaloil	Geranium synthetic oil	Geranium natural Oil 75 µl &DMSO25 µl	Geranium synthetic Oil 75 µl &DMSO25µl	Antifungal agent
Aspergillus niger	12.00±0.50[a]	6.00±0.10[a]	18.00±0.50[a]	10.96±0.35[a]	10.00±0.20[a] (Amphotericin B)
Aspergillus flavus	9.03±0.25[b]	5.00±0.10[b]	4.00±0.00[b]	7.03±0.15[b]	11.03±0.25[b] (Amphotericin B)
Candida albicans	6.96±0.35[c]	5.03±0.05[b]	4.00±0.10[b]	6.03±0.15[c]	12.00±1.00[c](Fluconazole)
Candida tropicalis	9.10±0.36[b]	6.03±0.15[a]	4.06±0.11[b]	7.00±0.20[b]	10.50±0.50[a](Fluconazol
Candida kefyr	7.03±0.15[d]	4.03±0.05[c]	4.00±0.10[b]	6.00±0.20[c]	9.96±0.45[d](fluconazole)

The values are represented as the Mean ± SD of two essential oils. These essential oils have significant effect at 0.05 levels

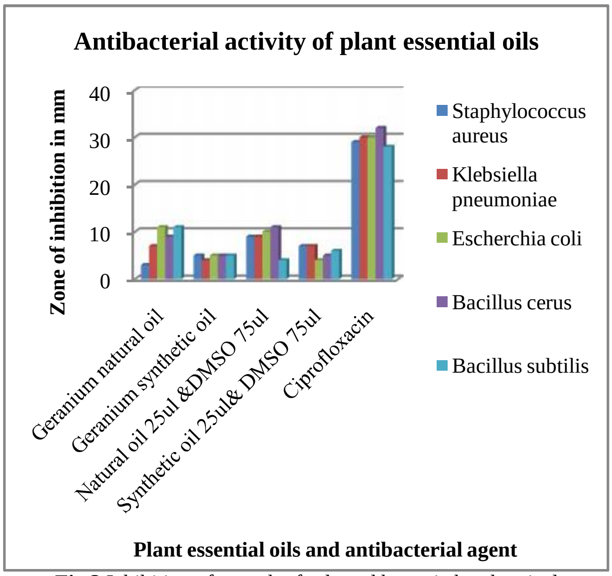

Fig 3 Inhibition of growth of selected bacteria by chemical compoundsof two essentialoils

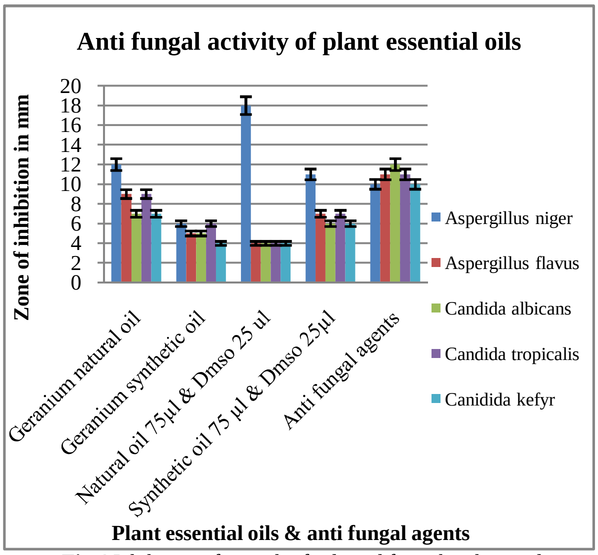

Fig 4 Inhibition of growth of selected fungi by chemical compoundsoftwo essentialoils

Table 7

Microorganisms	Geranium naturaloil	Geranium synthetic oil	Geranium natural Oil 50 µl &DMSO50µl	Geranium synthetic Oil 50 µl &DMSO50 µl	Antifungal agent
Aspergillus niger	14.00±0.50[a]	11.93±0.40[a]	8.00±0.20[a]	11.10±0.36[a]	4.00± 0.00[a] (Amphotericin B)
Aspergillus flavus	8.00±0.20[b]	3.00±0.00[b]	7.00 ±0.20[b]	5.00±0.10[b]	3.96±0.05[b] (Amphotericin B)
Candida albicans	7.03 ±0.15[c]	4.00±0.00[c]	6.00±0.10[c]	8.03± 0.15[c]	17.00±1.00[c](Fluconazole)
Candida tropicalis	12.00±0.50[d]	6.03±0.25[d]	11.03±0.55[d]	4.00±0.00[d]	19.00±1.00[d](Fluconazole)
Candida kefyr	14.00±0.50[a]	6.03±0.15[d]	11.10±0.36[d]	7.03±0.15[e]	20.00±1.00[e](Fluconazole)

Table 8

Microorganisms	Geranium naturaloil	Geranium synthetic oil	Geranium natural Oil 25 µl &DMSO75 µl	Geranium synthetic Oil 25 µl &DMSO75µl	Antifungal agent
Aspergillus niger	14.00±0.50[a]	11.06±0.30[a]	9.03±0.25[a]	7.03±0.15[a]	7.96± 0.25[a] (Amphotericin B)
Aspergillus flavus	11.96±0.45[b]	10.10±0.26[b]	14.96± 0.45[b]	10.96±0.45[b]	9.10±0.36[b] (Amphotericin B)
Candida albicans	8.03±0.15[c]	5.03±0.15[c]	7.06±0.20[c]	4.00± 0.00[c]	18.00±1.00[c](Fluconazole)
Candida tropicalis	7.03±0.15[d]	4.00±0.00[d]	5.03±0.15[d]	7.03±0.25[a]	19.00±1.00[d](Fluconazole)
Candida kefyr	10.93±0.40[e]	7.03±0.25[e]	5.03 ±0.15[d]	3.00±0.00[d]	21.00±1.00[e](Fluconazole)

In our investigation it has been found that geranium essential oil is effective against bacterial and fungal strains with different mechanisms of drug resistance. Geranium oil can be applied not only in thetreatment of dysentery, urinary tract and skin infections, but also in inflammation of the mouth, larynx, pharynx caused by bacterial and fungal pathogens. It can be used as an effective air disinfectant and as an additive to

Synergy of action essential oils with antibiotics and chemotherapeutics presents an opportunity for significant reductions of therapeutic doses, reduction of the adverse effects of antibiotic therapy and prevention of antibiotic-resistant strain formation. Because of the therapeutic problems associated with particularly resistant strains, essential oils can be useful in fighting against the microflora provoking hospitalacquired infections. (Dorian *et al*., 2009) have tested the antibacterial activity of geranium essential oil against bacterial and fungal pathogens.

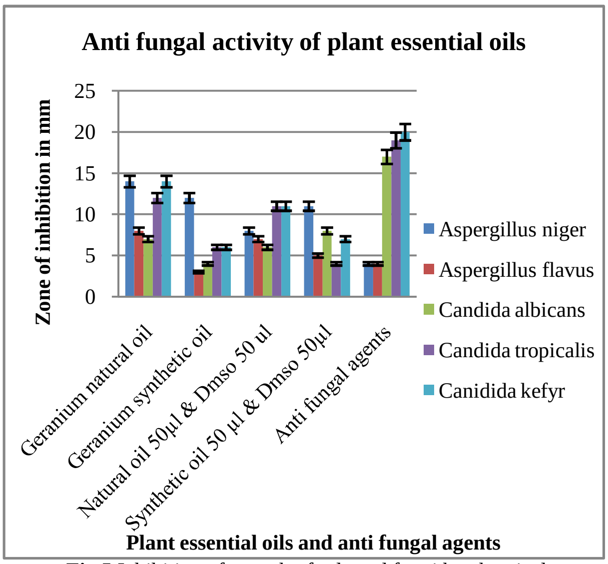

Fig 5 Inhibition of growth of selected fungi by chemical compoundsoftwo essentialoils

An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable . Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death . Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria. In the present study geranium oils were found to be equally effective against both gram-positive and gram negative organisms.

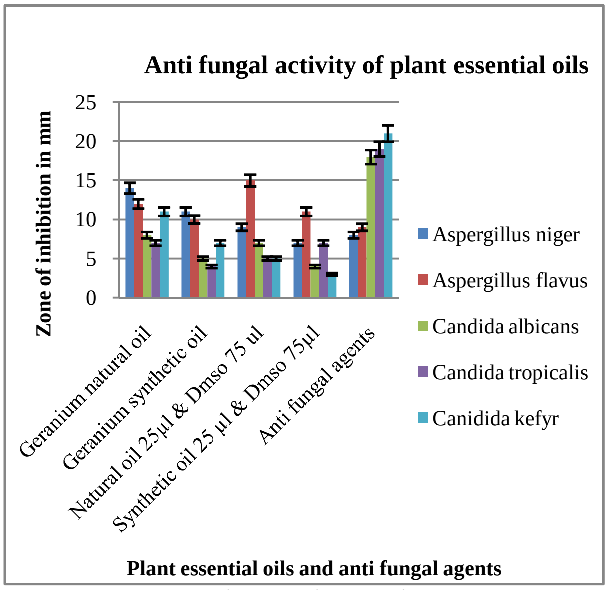

Fig 6 Inhibition of growth of selected fungi by chemical compoundsoftwo essentialoils

The observed activity showed that geranium oil have better as a good potential for use as antimicrobial agents and natural preservatives in different products.

However, further studies are needed to explore the efficiency of suitable concentration and to examine how the combined product exhibit antimicrobial activity. More studies should also confirm the safety of these essential oils and introduce the main compound responsible for antimicrobial activity.

CONCLUSION

Essential oils obtained from leaves, stems, and flowers of different plant species 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 essential oil from *P. graveolens* natural and syntheticshowed good antibacterial and anti-fungal activities and could be used in further pharmacological and phytochemical analysis. Additional studies *in vitro* and clinical trials would be needed to further characterize the active principles and evaluate the potential activity of these oils.

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