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STUDY ON MIC, MBC AND MFC ACTIVITY OF DIFFREENT EXTRACTS OF PELARGONIUM GRAVEOLENS

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INTRODUCTION

Resistance of human and food spoilage pathogens to antimicrobial agents and the side effects of chemical agents or preservative for human are caused for finding natural new antimicrobial agents, especially among the medicinal plants. This review introduces the methods that are used for antimicrobial evaluations and synergistic activities and the antimicrobial potential of some Indian medicinal plants. In recent years, the appearance of antibiotic resistant bacteria and fungi to antimicrobial agents has been an important issue for researchers. This resistance to antibiotics increases the morbidity rate in communities (Mazel and Davies, 1999). Because of adverse effects of chemical antibiotics and the resistant microorganisms, the scientists have interested in new sources of antimicrobial agent especially among medicinal plants. For a long time, some of plants have been medicinal or food values for humans and is used for treatment of human ailments (Cowan, 1999). Essential oils and plant extracts based on ethno medicinal uses are potential sources of new antimicrobial compounds against microbial strains. The combined use of plant extracts or essential oils and antibiotics are useful in decreasing drug resistant problems (Mahboubi and Ghazian Bidgoli, 2010). There are some medicinal plant species that are used by Indian people. Some of these plants are screened for these antimicrobial activities.

In this review, at first, we explain the methods that usually are used for antimicrobial evaluation and their synergistic activity with ordinary antibiotics, and then introduce some Indian medicinal plants with antimicrobial activities against food spoilage and human pathogenic bacteria and fungi. *Pelargonium graveolens* is a member of *Geraniaceae* family that is commercially cultivated for its essential oil. It is a traditional remedy for wounds, abscesses, fever, colic, nephritis, suppression of urine, cold, sore throat, hemorrhoids and gonorrhea. The essential oil of *P. graveolens* and its main components, geraniol and citronellol exhibited strong antimicrobial activity against *C. albicans*

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(Mahboubi *et al.,* 2008), *P. aeruginosa* (Mahboubi *et al.*, 2006), *A. niger, A. flavus* (Shin, 2003), *Tricophyton* spp (Shin and Lim, 2004), *Penicillium chrysogenum* (Yang and Clausen, 2007), *S. aureus, Streptococcus pneumonia, Escherichia coli, Klebsiella pneumonia* (Mativandlela *et al.,* 2006). Geraniol inhibited the growth of *C. albicans* and *Saccharomyces cervisiae* (Bard et al., 1998). Geraniol enhances the rate of potassium leakage out of whole cell and increases *C. albicans* membrane fluidity. Synergistic interaction between *P. graveolens* essential oil and amphotericin B is reported (Rosato *et al.,* 2008).

Materials and methods

Plant material

P. graveolens leaves were collected from Tirumala hills AndhraPradesh.

Preparation of plant organic extracts

A portion of dried leaves (100 g) of P. *graveolens* was placed in a soxhlet apparatus. Extraction was performed with 500 ml of an appropriate solvent with increased polarity for 24 h at 95^0C temperature not exceeding the boiling point of the solvent. The extract was filtered through a 45 µm filter paper and concentrated under vacuum. In this experiment three solvents were used: *n*-hexane, ethyl acetate and methanol. The resulting three solutions were concentrated *in vacuoum* to dryness to give n- hexane extract HE (4 g), ethyl acetate extract EtOAcE (10 g) and methanol extract MeOHE (12 g). The remaining residue was extracted by water infusion and lyophi lised to give water extract WE (15 g) and the resulting powder material was stored until tested. The stock solutions were kept at 4°C.

Essential oil extraction

The oil extraction was obtained from 0.5 kg fresh plant by steam distillation during 3 h using a Clevenger-type apparatus. The aqueous phase was extracted with dichloromethane (3 x 50 ml) and dried with anhydrous sodium sulphate. For the determination of the procedure yield, the solvent was evaporated using a rotavapory vacuum evaporator to afford 2.5 g. The resulting essential oil (EO) was stored at 4°C.

Antimicrobial activity

Test microorganism were obtain from IMTECH, Chandigarh. They included Gram- positive bacteria: *Bacillus subtilis* ATCC *6633, Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Entero- coccus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880, *Listeria monocytogenes* ATCC 2132 and Gram-negative bacteria: *Salmonella enterica* ATCC 1244, *Klebsiella pneumoniae* ATCC 10031.

The following *fungal strains* were also tested: *Aspergillus niger* ATCC 16404, *Aspergillus flavus* ATCC 9643, *Fusarium graminearum* ATCC 201828, *Fusarium oxysporum* ATCC 7601, *Rhizopus nigricans* ATCC 6227, and *Alternaria alternata* ATCC 6663.

The bacterial strains were cultivated in Muller-Hinton agar (MH) (Oxoid Ltd, UK) at the appropriate temperature for each strain at 37°C and fungi were cultured on Potatoes Dextrose agar (PDA) medium at 28°C. Working cultures were

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prepared by inoculating a loopful of each test bacteria in 3 ml of Muller-Hinton broth and were incubated at 37°C for 12 hours. For the test, final inoculums concentrations of 10^6 CFU/ml bacteria were used. Fungal spore suspensions were collected from the surface of such fungal colonies by gentle scraping with a loop and suspended in 10 ml Potato Dextrose broth (PDB).

This suspension was mixed vigorously by vortexing for 15-20 min. The spore suspension stock was diluted to obtain a concentration of 10^6 spores/ml (measured by Malassez blade).

Agar diffusion method

The antimicrobial activity of the *P. graveolens* was evaluated by means of agar-well diffusion assay according to Guven *et al.* (2006) with some modifications. Fifteen milli- litres of the molten agar (45°C) were poured into sterile petri dishes (0 90 mm). Working cell suspensions were prepared and 100 µl were evenly spreaded onto the surface of the agar plates of Mueller-Hinton agar for bacteria, or potatoes dextrose agar medium for fungi. Once the plates had been aseptically dried, 06 mm wells were punched into the agar with a sterile Pasteur pipette. The *P. graveolens oils* and extracts were dissolved in dimethylsulfoxide/water (1/1) and sterile water to a final concentration of 50 mg/ml. Thus, 50 µl were placed into the wells and the plates were incubated at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. Gentamicin (15 µg/ wells), Amphotericin B (20 µg/wells) and DMSO served as positive and negative control. Antimicrobial activity was evaluated by measuring the diameter of circular inhibition zones around the well. Tests were performed in triplicate.

Determination of MIC and MFC

Minimum inhibitory concentrations (MICs) of *P. graveolens* were determined according to Gulluce *et al.* (2007) against a panel of test microorganisms representing different species of different ecosystems. The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 µl. A stock solution of the *P. graveolens* (50 mg/ml) was prepared in dimethyl- sulfoxide/water (1/9). The inhibitory activity of the EO and organic extracts was properly prepared and transferred to each well in order to obtain a twofold serial dilution of the original sample and to produce the concentration range of 0.039-10 mg/ml. To each test well 10 µl of cell suspension were added to final inoculum concentrations of 10^6 CFU/ml for bacteria and 10^5 spores/ml for fungus. Positive growth control wells consisted of bacteria or fungi only in their adequate medium. Dimethylsulfoxide/water (1/9) was used as negative control. The plates were then covered with the sterile plate covers and incubated at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. The MIC was defined as the lowest concentration of the total essential oil at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 µl of Thiazolyl Blue Tetrazolium Bromide (MTT), indicator solution (0.5 mg/ml) dissolved in sterile water were added to the wells and incubated at 37°C for 30 min. The colourless tetra- zolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms. Where microbial growth was inhibited, the solution in the well remained clear after incubation with MTT. The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of 10 µl in Potatoes Dextrose agar (PDA) plates and incubated for 72 h at 28°C. The lowest concentration with no visible growth was defined as the MFC, indicating > 99.5% killing of the original inoculum. DMSO and etha nol were used as a negative control.

The determinations of MIC, MBC and MFC values were done in triplicate.

Results and discussion

Antibacterial activity

Plant extracts are widely claimed to have a broad- spectrum antibacterial activity and are considered as a main source for the search of lead compounds. The antimicrobial activities of *P. graveolens* essential oil and extracts against the tested microorganisms were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, MIC and MBC values. The results are given in Table 1. Among the tested extracts, only EtOAcE and MeOHE extracts exhibited an antimicrobial activity. HE and WE remained inactive in the range of the used concentration (4 mg/wells). Active extracts showed a potent antimicrobial activity against both Gram-positive and Gram-negative bacteria. The inhibition zone diameters and MIC values were in the range of 17-27 mm and 0.039-1.25 mg/ml for the ethyl acetate extract and 12-20 mm and 0.0782.5 mg/ml for the methanol one, respectively. Compared to the antibacterial activity of MeOH extract, and of the EtOAc showed a significant one ($p < 0.05$). Negative control did not show any inhibitory effect against the tested bacteria.

Also, our results showed that the essential oil of *P. graveolens* had great potential for antimicrobial activity against a panel of microorganisms. The maximum inhibition zone diameters and MIC values for bacterial strains, which were sensitive to the EO, were in the range of 13-26 mm and 0.312-10 mg/ml, respectively (Table 1).

On the basis of inhibition zone diameters, MIC and MBC values, *Staphylococcus aureus* was more sensitive to the EO than the other Gram positive bacteria with inhibition zone diameter of 24 mm and MIC and MBC values of 0.312 and 0.625 mg/ml respectively. Our results are in good agreement with the findings of Hussain (2010) who reported that Gram positive bacteria are more sensitive to plant essential oils than Gram negative bacteria, especially *E. coli* Kordali *et al.* (2005). The resistance of Gram negative bacteria against essential oils has been attributed to the presence of a hydrophilic outer membrane containing a hydrophilic polysaccharide chain, which acts as a barrier hydrophobic essential oil. The intense antimicrobial properties of essential oils from the leaves of *P. graveolens* was suspected to be associated with their high contents of oxygenated monoterpene appeared more active against the tested Gram positive than Gram negative bacteria. This result was in agreement with many studies realized on other plant species like *E. robusta, E. alba, E. camadulensis, E. citriodora, E. globulus, E. saligna,* Kordali *et al.* (2005).

Table 1. Determined MIC, MBC and MFC values of P. *graveolens* organic extracts and essential oil against bacterial species and fungal strains

Bacteria	Extracts	EtOAcE		MeOHE		EO	
Gram positive		MIC	MBC	MIC	MBC	MIC	MBC
Bacillus subtilis ATCC 6633		0.156	5	0.078	0.156	5	5
Bacillus cereus ATCC 14579		0.039	20	0.156	20	10	20
Staphylococcus aureus ATCC 25923		0.625	20	2.5	10	0.312	0.625
Staphylococcus epidermis ATCC 12228		0.156	0.312			5	10
Enterococcus faecalis ATCC29212		1.25	10	0	0	2.5	20
Micrococcus luteus ATCC1880		0.312	20	2.5	10	10	10

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Listeria monocytogenes ATCC 2132	0.156	0.625	2.5	5	2.5	10
Gram negative						
Salmonella enterica ATCC 1244	0.078	2.5	0.312	5	5	5
Klebsiella pneumoniae ATCC 10031	0.312	5	0.312	10	10	10
Fungal strains	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus niger ATCC 16404	0.312	2.5	1.25	20	0.625	5
Aspergillus flavus ATCC 9643	0.625	5	2.5	20	1.25	10
Fusarium graminearum ATCC 201828	1.25	10	0.625	5	0.312	5
Fusarium oxysposium ATCC 7601	1.25	5	0.312	2.5	0.156	5
Alternaria alternata ATCC 6663	0.625	5	1.25	10	0.625	5
Rhizopus nigrcans ATCC 6227	1.25	20	1.25	20	0.	10

Values are expressed on mg/ml.
MIC: Minimum inhibitory concentrations.
MBC: Minimum bactericidal concentrations.
MFC: Minimum fungicidal. concentrations.
EtOAeE: ethyl aeetate extract.
MeOHE: methanol extract.
EO: essential oil.

Furthermore, EtOAc and MeOHE of *P. graveolens* showed a potent inhibition for *B. subtilis, L. monocy- togenes* and *S. enterica* with MIC of 0.156 and 0.078 mg/ml respectively. Infections caused by these bacteria, specially those with multi-drugs resistance, are among the most difficult to treat with conventional antibiotics. In the current study, the growth of *B. subtilis* was remarkably inhibited by the ethyl acetate extract of *P. graveolens* (IZ = 27 mm). These results show that *P. graveolens* EtOAc organic extract can be used to minimize problems of drug resistance and protect foods against multiple pathogenic bacteria. Our data suggest that antibacterial activities might be related to the phenolic compounds found in the EtOAC and MeOH extracts.

Antifungal activity

Contamination by *Aspergillus, Fusarium* and *Alternaria* species especially with their respective mycotoxins is considered as a challenge for the pharmaceutical and food industries. The current study reports the capacity of the crude extracts and essential oil of *P. graveolens* to control *Aspergillus sp., Fusarium sp.* and *Alternaria alternata* strains. Among the tested extracts, only MeOH and EtOAc exhibited an antifungal activity.

Results showed a strong inhibitory effect of EtOAc on the growth of *A. niger* and *A. flavus* with inhibition zone diameters of mm and MIC values of 0.312 and 0.625 mg/ml respectively (Table 1). Also, the *P. graveolens* EO exhibited an antifungal activity against *Fusarium* and *Aspergillus* sp. such as *A. niger* and *A. flavus* which are responsible for food spoilage. The maximum inhibition zone diameters were 14 - 34 mm and MIC values ranged from 0.078 to 1.25 mg/ml (Table 1). The inhibition zone of the EO against *Aspergillus* sp. was recorded as 18-22 mm and the minimum inhibition concentration values were 0.625-1.25 mg/ml. Essential oil is a complex mixture of compounds with low molecular weights and the antimicrobial effect of total oil is related to one or a few principles

To the best of our knowledge, this is the first study providing data on the antifungal activity of the essential oil and organic extracts of *P. graveolens* plants evaluated against a wide range of fungal strains. Findings revealed that the extracts of this aromatic plant could be useful as an alternative antimicrobial agent in natural medicine for the treatment of many infectious diseases.

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Conclusion

P. graveolens is a medicinal and edible herb growing in India and used for a long time in Mediterranean cuisine, not only to improve or modify food flavor, but also to avoid its deterioration. We also investigated the antimicrobial activities of the essential oil and organic extracts from this herb. The ethyl acetate extract of *P. graveolens,* revealed a very important *in vitro* antibacterial activity on the studied bacteria, confirmed by low minimum inhibitory concentrations (MIC). It can therefore be used as a natural antimicrobial agent for the treatment of several infectious diseases. Furthermore studies are needed for the purification of the methanol extract and the identification of active molecules.

References

1. Ardestani A, Yazdanparast R: Antioxidant and free radical scavenging potential of *achillea santolina* extracts. *Food Chem* 2007, 104:21-29.
2. Barouh N, Abdelkafi S, Fouquet B, Pina M, Scheirlinckx F, Carriere F, Villeneuve P: Neutral lipid characterization of non-water-soluble fractions of carica papaya latex. *Journal of American Oil Chemist Society* 2010, 87:987-995.
3. Ben Hsouna A, Culioli MG, Blache Y, Jaoua S: Antioxidant constituents from *lawsonia inermis* leaves: isolation, structure elucidation and antioxidative capacity. *Food Chem* 2011, 125:193-200.
4. Ben Hsouna A, Trigui M, Ben Mansour R, Mezghani Jarraya R, Damak M, Jaoua S: Chemical composition, cytotoxicity effect and antimicrobial activity of *ceratonia siliqua* essential oil with preservative effects against *Listeria* inoculated in minced beef meat. *Int J Food Microbiol* 2011, 148:66-72.
5. Boukhris M, Simmonds MSJ, Sayadi S, Bouaziz M: Chemical composition and biological activities of polar extracts and essential oil of rose- scented geranium, *Pelargonium graveolens. Phytother Res* 2012, doi:10.1002/ptr.4853.
6. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N: Antioxidant activity of some Algerian medicinal plants extracts containing Phenolic compounds. *Food Chem* 2006, 97:654-660.
7. Fendri I, Chaari A, Dhouib A, Jlassi B, Carriere F, Sayadi S, Abdelkafi S: Isolation, identification and characterization of a new lipolytic pseudomonas sp., strain AHD-1, from Tunisian soil. *Environ Technol* 2010, 31:87-95.
8. Fusco D, Colloca G, Lo Monaco MR, Cesari M: Rationale for antioxidant supplementation in *sarcopenia. J Clin Interv Aging* 2007, 2:377-387.
9. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H: Antimicrobial and antioxidant properties of the essential oils and methanol extract from *mentha longifolia* L. ssp. *Longifolia. Food Chem* 2007, 103:1449-1456.
10. Gupta S, Prakash J: Studies on indian green leafy vegetables for their antioxidant activity. *Plant Foods Hum Nutr* 2009, 64:39-45.
11. Guven K, Yucel E, ^etintaj F: Antimicrobial activities of fruits of *crataegus* and *pyrus* species. *Pharm Biol* 2006, 44:79-83.
12. Hertog MGL, Hollman PCH, Van de Putte B: Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal Agricultrual Food Chemistry* 1993, 41:1242-1246.

13. Hussain AI, Anwar F, Shahid M, Ashraf M, Przybylski R: Chemical composition, antioxidant and antimicrobial activities of essential oil of spearmint *(mentha spicata* L.) from Pakistan. *Journal Essential Oil Research* 2010, 22:78-84.
14. Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal BB: Natural products as a gold mine for arthritis treatment. *Curr Opin Pharmacol* 2007, 7:344-351.
15. Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A: Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. *J Agric Food Chem* 2005, 53:1408-1416.
16. Loizzo MR, Tundis R, Conforti F, Saab AM, Statti GA, Menichini F: Composition and **a**-amylase inhibitory effect of essential oils from *cedrus libani. Fitoterapia* 2007, 78:323-326.
17. MAHBOUBI, M. - FEIZABADI, M. M. - HAGHI, G. - HOSSEINI, H. 2008. Antimicrobial activity and chemical composition of essential oil from *Oliveria decumbens* Vent. In *Iranian Journal of Medicinal and Aromatic Plants,* vol. 24, p. 56-65.
18. MAHBOUBI, M. - SHAHCHERAGHI, F. - FEIZABADI, M. M. 2006. Bactericidal effects of essential oils from clove, lavender and geranium on multi-drug resistant isolates of *Pseudomonas aeruginosa.* In *Iranian Journal of Biotechnology,* vol. 4, p. 137- 40.
19. Mativandlela SPN, Lall N, Meyer JJN: Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) *(Geraniaceae)* root extracts. *South African Journal of Botany* 2006, 72:232-237.
20. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyck M, Cazin M, Cazin JC, Bailleul F, Trotin F: Phenolic compounds and antioxidant activities of buckwheat (fagopyrum esculentum moench) hulls and flour. *J Ethnopharmacol* 2000, 72:35-42.
21. 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. In *Phytomedicine,* vol. 15, p. 635-638.
22. Saraswathi J, Venkatesh K, Baburao N, Hilal MH, Roja Rani A: Phytopharmacological importance of *pelargonium* species. *Journal of Medicinal Plants Research* 2011, 5:2587-2598.
23. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG: Composition and antimicrobial activity of essential oils from aromatic plants used in brazil. *Braz J Microbiol* 2004, 35:275-280.
24. SHIN, S. - LIM, S. 2004. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. In *Journal Applied Microbiology,* vol. 97, p. 1289-1296.
25. SHIN, S. 2003. Anti *Aspergillus* activities of plant essential oils and their combination effects with ketoconazole or amphotericin B. In *Archives of Pharmacal Research,* vol. 26, p. 389393.
26. Shojaee-Aliabadi S, Hosseini H, Mohammadifar MA, Mohammadi A, Ghasemlou M, Mahdi Ojagh S, Marzieh Hosseini S, Khaksar R: Characterization of antioxidant-antimicrobial **K**-Carrageenan films containing satureja hortensis essential oil. *Int J Biol Macromol* 2012, In Press.
27. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M: Antimicrobial and antioxidant activities of essential oil and various extracts of salvia tomentosa miller *(lamiaceae). Food Chem* 2005, 90:333-340.

28. YANG, V. W. - CLAUSEN, C. A. 2007. Antifungal effect of essential oils on southern yellow pine. In *Biodeterioration andBiodegradation,* vol. 59, p. 302-306. ZARGARI, A. 1995. Medicinal Plants. Tehran University Press, Tehran.
29. Yen G, Wu S, Duh P: Extraction and identification of antioxidant components from the leaves of mulberry (morus alba L.). *Journal Agricultrual Food Chemistry* 1996, 44:1687-1690.