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Antimicrobial activity of the essential oils of three species of *Pogostemon*

J.E. Thoppil[1]*, A. Tajo[1], J. Minija[1], M.J. Deena[1], K. Sreeranjini[1], L. Leeja[1], M. Sivadasan[2] and A.H. Alfarhan[2]

[1]Cell and Molecular Biology Division, Department of Botany, University of Calicut, Calicut–673 635, India

[2]Department of Botany and Microbiology, College of Science, King Saud University, P.B. No. 2455, Riyadh–11451, Kingdom of Saudi Arabia

*Corresponding Authors Email : jethoppil@gmail.com

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Abstract

Antimicrobial studies on 7 strains of bacteria and 8 strains of fungi using disk diffusion assay, revealed potential activities of crude essential oils in *Pogostemon benghalensis, P. purpurascens* and *P. vestitus.* Essential oils produced highest inhibition zones against *Staphylococcus aureus* (39.33, 37.33 and 35.67 mm for *P. benghalensis* essential oil) and *Candida albicans* (34.33, 26.33 and 17.67 mm for *P. purpurascens* essential oil) among bacteria and fungi respectively, when compared with pure reference standards (35 mm for Gentamycin sulphate (40 mg ml^{-1}) against *S. aureus* and 30.33 mm for Nystatin [50 IU] against *C. albicans*). Results also indicated the existence of potential antimicrobial activity of *Pogostemon* essential oils against other microorganisms *viz*., *Proteus vulgaris*, *E. coli* and *Aspergillus parasiticus*. Leaf essential oils of *P. purpurascens* and *P. benghalensis* can be considered as a new source for developing local antifungal and antibacterial agents. An attempt was made to highlight the promising plant species for further investigation which leads to drug development.

Key words

Antibacterial, Antifungal, Antimicrobial, Lamiaceae, *Pogostemon*

Introduction

Foods contaminated with pathogens are often identified as primary sources of food-borne diseases in humans. The survival and growth of microorganisms in food products may lead to their spoilage and quality deterioration (Celiktas *et al.,* 2007).

Western Ghats provide a rich source of aromatic and medicinal plants, having natural products *viz*., essential oils and oleo-resins with biological activities (Parrotta, 2001). Scientific interest in phytomedicine has burgeoned due to increased efficacy of new plant-derived drugs, emerging interest in natural products and increasing concerns about the side effects of conventional medicine (Senthilkumar *et al*., 2010). In this context, a systematic antimicrobial screening of essential oils of folk herbs and medicinal plants may result in the discovery of novel and effective antimicrobial compounds (Janovská *et al.,* 2003).

Essential oils are considered as one of the potential sources for screening of anticancer, antimicrobial, antioxidant and free radical scavenging agents (Celiktas *et al*., 2007; Hussain *et al*., 2008). Essential oils have shown potential as anti-bacterial agents, disinfectants, anti-fungal agents, insecticides and as herbicides (Skocibusic *et al*., 2006; Bozin *et al.,* 2006; Maksimovic *et al*., 2007; Van Vuuren *et al*., 2007). The most thoroughly examined are antimicrobial properties of essential oils, which in many ways are better than those exhibited by synthetic antibiotics due to their wider spectrum of activity. In particular, antimicrobial activities of essential oils have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Bozin *et al.,* 2006). Plant essential oils and extracts have broad–spectrum activity against both Gram-negative and Gram-positive food-borne microbes (Celiktas *et al.,* 2007; Hussain *et al.,* 2008; Hussain *et al.,* 2010; Kotzekidou *et al.,* 2008). Bactericidal properties of essential oils may also be used for disinfecting air. Utilization of essential oils for microbial-free storage of raw and processed food has also been focused (Burt, 2004; Devlieghere *et al*., 2004; Holley and Patel, 2005).

Pogostemon benghalensis (Bengal pogostemon), *P. purpurascens* (Sangbrei) and *P. vestitus* are used to cure some ailments in the villages of southern Western Ghats in Kerala (India). *Pogostemon purpurascens* is used as a stimulant, anti-colic, anti-haemorrhagic, styptic, febrifuge, antidote to scorpion sting, snake bite, as a cure for burns, to clean wounds and for promoting granulation (CSIR, 1986). Whereas *P. benghalensis* is used as a stimulant, styptic, anti-colic, febrifuge and for wound healing. It is used to treat indigestion, diarrhoea, food poisoning, vomiting, stomach troubles, fever, cough, cold and typhoid fever. It also exhibits antiviral, antibacterial, antimycobacterial and antipyretic activities (Saikia and Khan, 2011). *Pogostemon vestitus* is used as a tonic to cure fever and cold (Samydurai *et al*., 2012).

Hence considering the aforesaid, this study aimed to authenticate the antimicrobial activity of essential oils obtained from the leaves of *Pogostemon benghalensis, P. purpurascens* and *P. vestitus,* against some selected bacteria and fungi.

Materials and Methods

Three species of *Pogostemon* Desf. *viz*., *P. vestitus* Benth., *P. purpurascens* Dalzell and *P. benghalensis* Kuntze collected from various parts of Western Ghats were grown in Botanical Garden, Department of Botany, University of Calicut. Plant voucher specimens were deposited (CALI 88422 – CALI 88424) in Herbarium of University. Leaves of these plants were collected during October – December 2011, washed and shade dried. Chopped and powdered raw materials (100g) were hydrodistilled in an all-glass Clevenger-type apparatus at 100 ºC for 4 hr to extract essential oils, according to the method outlined in the European Pharmacopoeia (Anonymous, 1996). The aromatic essential oils obtained were used for further investigation.

To test the antimicrobial activity, 7 strains of bacteria and 8 strains of fungi obtained from MTCC Gene Bank, Institute of Microbial Technology, Chandigarh, India were used (Table 1). Agar-based disk diffusion assay (Benson, 1990) was used to reveal the microbicidal potentialities of crude essential oils of *Pogostemon* species as well as their dilutions (1:1 and 1:2) in diethyl ether. Growth inhibition was evaluated after 48 hr and compared with those results obtained from the controls using Gentamycin sulphate and Nystatin (both SIGMA-ALDRICH) for bacteria and fungi respectively. Zone of growth inhibition for each microorganism was calculated.

Results and Discussion

Three species of *Pogostemon* studied were found to be blessed with moderate amounts of essential oil, with the maximum for *P. benghalensis* (0.6%), followed by *P. purpurascens* (0.35%) and *P. vestitus* (0.25%). Essential oils of these taxa were found most effective against *Staphylococcus aureus* among seven bacteria tested. Three species of *Pogostemon* also showed promising repellency against all the seven strains of bacteria studied. However, remarkable activity was noted against *Escherichia coli* and *Proteus vulgaris* when compared with the antibacterial activity of pure standard, gentamycin sulphate (Table 1). The efficacy of all the three essential oils decreased with increasing dilution using diethyl ether. However, the essential oil of *P. benghalensis,* as well as its dilution were found to be effective against *Staphylococcus aureus,* with an inhibition zone of 39.33, 37.33 and 35.67 mm respectively. This was found to be higher than that with the standard drug, gentamycin sulphate. *In vitro* and *in vivo* antibacterial studies conducted by Dai *et al*. (2012) proved the efficacy of *P. cablin* essential oil in treating dairy cows suffering with bovine mastitis caused by *E. coli* and *S. aureus*. Thus *Pogostemon* essential oil seem to be prospective antibacterial agents in controlling resistant pathogenic mammalian infections, especially multi-drug resistant pathogenic infections.

Essential oil obtained from three species of *Pogostemon* showed potential antifungal activity against eight strains of the fungi studied, more significantly, *Candida albicans* (Table 1). Moderately high activity was shown by *P. purpurascens* against *Aspergillus parasiticus,* with an inhibition zone of 30.33, 25.33 and 20.67 mm respectively, when compared with the antifungal activity of pure standard, Nystatin. An earlier study had effectively proved the anti-Candida activity of essential oil of *P. parviflorus* (Najafi and Sadeghi-Nejad, 2011). *Candida* species are harmless commensal yeast-like fungi in healthy humans, which can cause superficial as well as life-threatening systemic infections under immune-compromised situations (Jain *et al*., 2010). Furthermore, long-term treatments with commonly used antifungal drugs, such as polyenes and azoles, have toxic effects and result in drug resistance (Singh *et al*., 2006).

Recent researches had revealed the mechanism of action of plant essential oils on bacteria and fungi (Bakkali *et al*., 2008). Being lipophiles, essential oils pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. In bacteria, permeabilization of membrane is associated with loss of ions and reduction of membrane potential, collapse of proton pump and depletion of ATP pool. Damage to cell wall and membrane can lead to the leakage of macromolecules and to lysis (Oussalah *et al*., 2006). In eukaryotes (including fungi), essential oils can provoke depolarization of mitochondrial membranes by decreasing membrane potential, affect ionic Ca^{++} cycling and other ionic channels and reduce pH gradient, affecting proton pump and ATP pool. They change the fluidity of membranes, which become abnormally permeable, resulting in leakage of radicals, cytochrome C, calcium ions and proteins as in case of oxidative stress and bioenergetic failure.

Table 1 : Antimicrobial activity of crude and diluted essential oils of *Pogostemon* spp.

Micro organisms (MTCC No.)	Zone of inhibition (mm) of microbial growth *										
	Essential oil and its dilutions in diethyl ether									Standards	
	Pogostemon vestitus			*P. purpurascens*			*P. benghalensis*			Gentamycin sulphate (40 mg/ml)	Nystatin (50 IU)
	1:0	1:1	1:2	1:0	1:1	1:2	1:0	1:1	1:2		
Bacillus megaterium (428)	31.67 ±2.52	29.00 ±1.00	26.00 ±1.73	35.67 ±1.15	33.67 ±2.08	29.00 ±2.65	34.00 ±2.00	31.67 ±2.08	30.33 ±2.08	45 ±3.00	
B. subtilis (121)	29.33 ±3.05	26.67 ±1.53	26.00 ±2.65	38.00 ±2.65	33.00 ±1.00	31.33 ±1.53	36.00 ±1.73	34.33 ±2.52	31.67 ±2.89	48 ±2.00	
Escherichia coli (118)	30.00 ±2.65	27.33 ±1.53	26.67 ±2.31	35.00 ±2.00	26.67 ±2.52	26.33 ±1.53	31.00 ±2.65	30.33 ±2.08	30.33 ±3.05	28.67 ±1.53	
Staphylococcus aureus (3160)	36.67 ±1.53	30.33 ±2.52	28.00 ±1.00	38.00 ±3.61	33.33 ±1.53	31.33 ±2.08	39.33 ±1.53	37.33 ±2.08	35.67 ±2.08	35 ±1.00	
Xanthomonas campestris (2286)	33.67 ±2.52	31.33 ±3.05	28.00 ±1.73	38.00 ±2.65	33.67 ±1.15	31.33 ± 3.79	35.67 ±1.53	31.67 ± 1.53	28.00 ±3.60	53.33 ±2.52	
Pseudomonas aeruginosa (424)	27.33 ±2.52	25.67 ±1.53	25.67 ±2.52	29.33 ±1.53	25.00 ±1.73	23.33 ±3.05	28.00 ±3.00	26.67 ±1.15	25.67 ±2.08	40.33 ±1.53	
Proteus vulgaris (426)	29.33 ±2.08	27.33 ±2.08	25.67 ±1.53	31.67 ±2.08	30.33 ±4.51	30.00 ±3.00	29.67 ±1.53	27.33 ±1.15	25.67 ±3.51	27.67 ±0.58	
Aspergillus niger (281)	31.33 ±3.05	21.33 ±3.05	17.33 ±2.52	36.33 ±1.53	30.33 ±4.04	28.00 ±3.61	31.33 ±2.52	28.00 ±3.61	24.00 ±2.00		38.33 ±2.52
A. parasiticus (2796)	28.67 ±3.05	22.33 ±2.08	19.33 ±1.15	30.33 ±3.05	25.33 ±2.08	20.67 ±3.79	28.67 ±3.05	26.33 ±1.53	23.33 ±2.08		29 ±3.00
Rhizopus oryzae (262)	26.33 ±2.52	22.33 ±1.53	19.00 ±2.65	28.33 ±3.21	22.67 ±1.53	19.67 ±1.53	22.67 ±2.52	19.00 ±2.00	16.67 ±1.53		30.67 ±0.58
Rhizoctonia oryzae-sativae (2162)	29.00 ±3.61	25.33 ±3.05	20.67 ±3.05	30.33 ±2.08	26.33 ±1.53	19.33 ±3.05	27.33 ±1.53	23.33 ±1.53	17.67 ±2.08		33.33 ±2.52
Fusarium solani (350)	34.33 ±1.53	27.67 ±2.52	22.33 ±2.52	38.33 ±2.52	36.33 ±2.52	22.33 ±2.52	32.33 ±2.08	28.33 ±1.53	20.33 ±3.05		40.67 ±1.53
Candida albicans (183)	32.33 ±2.08	24.33 ±2.52	19.67 ±2.31	34.33 ±3.05	26.33 ±2.89	17.67 ±2.31	32.33 ±2.52	27.00 ±2.00	18.67 ±2.52		30.33 ±1.53
Colletotrichum musae (2018)	25.33 ±2.08	20.67 ±2.08	17.67 ±1.53	28.67 ±3.21	23.67 ±1.53	21.33 ±2.52	26.33 ±2.08	18.67 ±1.53	16.33 ±2.08		30.67 ±2.52
Alternaria brassicicola (2102)	27.33 ±3.05	25.33 ±2.52	19.67 ±2.08	27.67 ±2.52	20.67 ±2.08	19.33 ±3.21	26.33 ±1.53	20.67 ±3.05	19.33 ±2.52		32.33 ±1.53

* Values represent mean ± standard deviation of 3 replicates including the diameter of the filter paper disk (16 mm)

Permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis (Armstrong, 2006).

This cytotoxic property is of great importance in the application of essential oils, not only against certain human or animal pathogens or parasites but also for the preservation of agricultural or marine products (Bakkali *et al.*, 2008). In the present investigation, it was found that the essential oils of *Pogostemon* species, especially, *P. purpurascens* and *P. benghalensis* showed highly significant microbicidal activity even higher than that of pure standards (Table 1). This emphasizes the superiority of these essential oils against commercial synthetic microbicides, stressing the need for further investigations using isolated and purified essential oil components. Both, the essential oils of *Pogostemon* as well as its dilutions may prove to be effective herbal protectants against a wide spectrum of pathogenic bacteria and fungi, since herbal microbicides are non-toxic and ecofriendly. Plant-based essential oils or organic extracts are well known to exhibit a wide range of biological activities but with low mammalian toxicity, less environmental effects and wide public acceptance (Paranagama *et al*., 2003). Thus *Pogostemon* essential oils may be used as antiseptic agents for preserving food.

To conclude, *Pogostemon* essential oils exerted remarkable microbicidal activity against all the microbes analyzed. The potent species, *viz*., *P. purpurascens* and *P. benghalensis* exerted significant repellency on pathogenic *Staphylococcus aureus* and *Candida albicans.* Since these plants are ubiquitous and their essential oils are environmentally non-persistent as well as safe for humans, they seem to be reliable and prospective candidates to be used as topical botanical-microbicides / antiseptics / therapeutics.

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