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Antimicrobial Properties of *Osmanthus fragrans* (Lour)

[1]Ashok Kumar and [2]Deepak Ganjewala
[1]Sardar Bhagwan Singh (PG) Institute of Biomedical Sciences and Research, Balawala, Dehradoon (UA), India
[2]School of Biotechnology, Chemical and Biomedical Engineering, VIT-University, Vellore-632 014 (Tamil Nadu), India

Abstract: The ethanol extract and essential oil of *Osmanthus fragrans* (Lour.) family (Oleaccae) were evaluated for antimicrobial action on *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi* and *Shigella dysentery* by using agar disc diffusion method. Essential oil have shown strongest inhibitory effect against *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhi*. Ethanol extract had less antimicrobial activity against the microorganisms tested. However, both essential oil and ethanol extract had no inhibitory effect against *Escherichia coli* and *Pseudomonas*. *Shigella dysentery* in particular was more susceptible for essential oil. Results have shown that essential oil had two fold more antibacterial activities as compared to that of ethanol extract. The minimum concentration of ethanol extract and essential oil used was 0.0625 mg mL^{-1}.

Key words: Antibacterial activity, minimum inhibition concentration, oleaceae, *Osmanthus fragrans*, essential oil

INTRODUCTION

The medicinal and aromatic plants have been used since ancient times for the treatment of human ailments and as natural food preservatives. Essential oils obtained from aromatic plants are occupying ever-increasing and widely varied significance owing to their specific recognition in pharmaceutical, flavor and perfumery industries (Guenther, 1950). The essential oils (monoterpenes) of a number of plants exhibit specific biological, pharmaceutical and therapeutical activities (Singh *et al*., 1989). *Osmanthus fragrans* (Lour.) family Oleaccae, native to China is valued for its delicate fruity-floral apricot aroma which is used in only the most expensive perfumes and flavors (Wang *et al*., 2006). In India it is found in high altitude areas in Kumaun region of Himalaya and it is commonly known as silang-shilang or silingi (Hussain and Virmani, 1992). Beta-ionone (10%) and beta-damascenone, theaspirane and theaspirone, megastigmadienone and -trienone and (+)-gamma-decalactone (4%) have been reported as major chemical constituent (Ohloff and Fragrances, 1994). The most defined work on *O. fragrance* has been carried in relation to germplasm resources, cultivar classification, chemical constituents and senescence and hardiness physiology (Liu and Xiang, 2003). Recently, Wang *et al*. (2006) have characterized the melanin from *O. fragrance* for their color value. However, there is no information regarding the pharmacological and medicinal significance. Therefore, the present study was undertaken to investigate the antimicrobial properties of *O. fragrance* found in Kumoun region of Himalya in India. In this study we have selected the ethanol extract and leaf essential oil for determining the antimicrobial activity against gram-negative and positive bacteria for the first time.

Corresponding Author: Dr. Deepak Ganjewala, School of Biotechnology, Chemical and Biomedical Engineering, VIT-University, Vellore-632 014 (Tamil Nadu), India
Tel: 91-0416-2243091, 91-99945-47146

MATERIALS AND METHODS

Plant Materials

Osmanthus fragrans (Oleaccae) plants were collected from Kumaun Regions of Himalayan range in India during the month of October-December 2003-2004 and taken immediately to the Sardar Bhagwan Singh (PG) Institute o f Biomedical Sciences and Research, Dehradoon and subjected to essential oil extraction. Leaves were oven dried till the constant weight at 65°C in an oven.

Ethanol Extract

One hundred milligram dried leaf powder was extracted twice with 20 mL ethanol and left at room temperature overnight. The ethanol was evaporated on a flash evaporator to dryness and kept at 4°C until use.

Essential Oil Isolation

Leaf essential oil was isolated by hydro-distillation using mini Clevenger (1928) apparatus.

Pharmacological Screening

Antimicrobial activity of ethanol extract and essential oil was determined against gram-positive (*Staphylococcus aureus Bacillus cereus*) and gram-negative (*Salmonella typhi*, *Shigella dysentery Escherichia coli*, *Pseudomaons species*) bacteria by using the disc-diffusion method (Smith *et al*., 2002). Ethanol extract was diluted in DMSO to 1:1, 1:2, 1:4, 1:6, 1;8 (v/v). Essential oil was diluted in diethyl ether. Twenty five microliter each of extract and essential oil was loaded onto sterile Whatman paper disc (7 mm) and oven dried at 37°C for 1 h to remove the presence of used solvent. After 24 h of incubation with impregnated discs, the plates were observed and zone of inhibition was measured. Ampicillin was used as the standard antibiotic (Fereshteh *et al*., 2005).

RESULTS AND DISCUSSION

Antimicrobial activities of ethanol extracts and essential oil are presented in Table 1 and 2. In general, essential oils have shown strongest inhibitory effect while ethanol extract had less antimicrobial activity against microorganisms tested. The results indicated that ethanol extract had only considerable antimicrobial activity against gram-positive (*S. aureus* and *B. cereus*) and gram-negative bacteria (*S. typhi*). However, the extract had no antimicrobial activity against other gram-negative bacteria (*S. dysentery*, *E. coli* and *Pseudomoas*).

Table 1: Antibacterial properties of *O. fragrans* leaf extract in ethanol against various microorganisms using agar disc diffusion method

	Concentration (μg mL^{-1})					
	100	50	25	12.5	6.25	Ampicillin
Microorganisms	Zone of growth inhibition (mm)					
Gram-positive						
Staphylococcus aureus	24	22	20	16	10	14
Bacillus cereus	19	18	16	14	14	14
Gram-negative						
Salmonella typhi	18	16	15	14	14	13
Shigella dysentery	18	00	00	00	00	10
Escherichia coli	00	00	00	00	00	00
Pseudomaons species	00	00	00	00	00	00

Table 2: Antibacterial properties of *O. fragrans* leaf essential oil against various microorganisms using agar disc diffusion method

	Concentration (μg mL^{-1})					
	100	50	25	12.5	6.25	Ampicillin
Microorganisms	Zone of growth inhibition (mm)					
Gram-positive						
Staphylococcus aureus	44	40	38	38	32	14
Bacillus cereus	36	35	35	21	18	14
Gram-negative						
Salmonella typhi	36	36	35	34	24	13
Shigella dysentery	44	44	42	34	32	10
Escherichia coli	00	00	00	00	00	00
Pseudomaons species	00	00	00	00	00	00

Essential oil have shown a potent antimicrobial activity against gram-positive *S. aureus* and *B. cereus*) as well gram-negative bacteria (*S. typhi* and *S. dysentery*) and in particular *S. dysentery* was more susceptible to essential oil as compared to ethanol extract. Similar to ethanol extract, essential oils had no inhibitory effect against gram-negative *E. coli* and *Pseudomas*.

The observed difference in antibacterial activity is expected due to variation in chemical compositions of ethanol extract and essential oil. The antimicrobial activity of *O. fragrance* ethanol extract may be attributed to chemical constituents and in particular of carotenoid derived materials which are all trans-beta-and alpha-Carotene and Neo-beta-carotene with large number of ionone derivatives and Theaspirane. Melanin, natural color pigments from *O. fragrance* seeds have recently been characterized (Wang *et al*., 2006). Natural melanin from plants or animals is reported for broad spectrum of biological activity and immuno-pharmaceutical properties (Barr, 1983; Lekiewicz, 1972).

Essential oil, the complex mixtures of the monoterpenes from a number of plants exhibited the specific biological, pharmaceutical and therapeutical activities (Singh *et al*., 1989). Though we have not investigated the essential oil composition of *O. fragrance* used in the study, however the antimicrobial properties of essential oil is expected since large number of chemical compounds viz., cis-3-hexenol, cis-3-hexenyl butyrate, cis-3-hexenyl propionate, cis-3-hexenyl benzoate, cis-3-hexenyl 2-hexenoate, nonanal, octanal, decanal, benzaldehyde, ethyl hexanoate and esters have been reported as essential oil constituents (Kaiser and Lamparsky, 1981). Further, the essential oils are hydrophobicity which enables them to a partition in the lipids of the bacterial cell membrane, disturbing the structures and rendering them more permeable (Oonmetta-aree *et al*., 2006). The ethanol extract and essential oil could not inhibit the growth of *E. coli* suggesting that these could not penetrate the outer membrane similar to galangal extract (Oonmetta-aree *et al*., 2006). The result has shown that the antibacterial activity of ethanol extract and essential oil was slightly dose dependent which increased with increase in concentration (dose). All of these results demonstrated that *O. fragrance* should be used for further studies about antimicrobial activity against other microorganisms with emphasis on identification of main component of extract and essential oil responsible for pharmacological properties.

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