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Antibacterial activity of essential oil of *Ocimum sanctum* L.

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Abstract

Antibacterial activity of *Ocimum sanctum* L. essential oil was evaluated against five human pathogenic bacterial species *E. coli*, *Klebsiella* sp., *P. mirabulus*, *P. aeruginosa* and *S. aureus*. by disc-diffusion method. Six mm discs were impregnated with 5 and 10 µl of undiluted essential oil and seeded over the plates aseptically having test microorganisms. The zones of inhibition were measured after 24 hours at 37 °C. The essential oil exhibited significant antibacterial activity against all the test pathogens, with maximum zone of inhibition against *S. aureus* (20.0 mm & 41.5 mm) and minimum against *E. coli* (10.2 mm & 17.8 mm) for 5 and 10 µl of essential oil, respectively. Similarly, the inhibition zones recorded in *P. mirabulus* were 15.1 mm & 26.0 mm, in *P. aeruginosa* 10.2 mm & 20.0 mm, in *Klebsiella* sp11.1 mm & 19.4 mm for two given concentrations of essential oil..

Key words: Antibacterial activity, disc-diffusion, essential oil, *Ocimum sanctum,* steam distillation.

Introduction

Essential oil is more or less volatile material isolated from an odoriferous plant of a single botanical species, commonly extracted by steam distillation (Sattar and Shahid, 1992). An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oil differs from the fatty or fixed oils both in composition and properties. Essential oils have no fixed structure as they are the mixture of different components including alcohols, phenols, aldehydes, ketones, acids, terpenes, hydrocarbons and esters etc (Kothari *et al*., 2005). Medical applications of these oils range from skin treatments to remedies for cancer (Prakash and Gupta, 2005). Essential oils are the heart and soul of everything we do, being used in perfumes, flavors, cosmetics, beverages, medicinal foods, paints, disinfectants, fungicides, smoking and condiments etc (Farooq *et al*., 2001).

Ocimum sanctum L. (Labiatae) is a strongly scented small annual herb, up to 18 inches tall and grows into a low bush and is commonly known as "holy basil", 'Tulsi' or 'Tulasi'. The small aromatic herb grows wild in Pakistan and widely cultivated in home and gardens. Different parts of the plant have been reported to be effective in wide spectrum of diseases (Singh *et al*., 1996). In several traditional medicinal systems, including Ayurveda, Roman, Greek, Sidha and Unani medicinal system, various therapeutic properties of *O. sanctum* have been mentioned. It has been reported to possess anti-carcinogenic, anthelmintic, anti-septic, anti-rheumatic, anti-stress and anti-bacterial properties (Godhwani *et al*., 1987; Singh and Majumdar, 1999). It is being used as a tonic for the treatment of nervous disorders, stress related headaches, migraines and allergies (Archana and Namasivayam, 2000). Due to peculiar essence of *O. sanctum* oil, used to clear the mind and relieve the intellectual fatigue, while giving clarity and mental strength. The oil is also administered for asthma, bronchitis, sinus infections, constipation, nausea, vomiting and cramp (Prakash and Gupta, 2002).The aim of present study was to screen the antibacterial efficacy of *O. sanctum* essential oil against both gram-positive and gram-negative strains

Materials and Methods

Plant material

Fresh leaves of five months old *O. sanctum* were collected from the Herbal Heritage Garden, Institute of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan. Leaves were washed under tap water and cut into small pieces to expose the oil sacs. Fragrant essential oil was extracted from these crushed leaves by steam distillation.

Extraction of essential oil

Sixty gram of fresh leaves of *O. sanctum* were subjected to steam distillation in a Dean and

Stark assembly (Sattar and Shahid, 1972) for three hours. Two layers were formed, upper organic layer of oil and lower aqueous layer of water. Lower aqueous layer was discarded and upper layer of *O. sanctum* oil was collected. The essential oil was further purified by solvent extraction using diethyl ether (25 ml). Bright yellow oil was further refined with Na_2SO_4 in order to remove traces of water if present. The essential oil sample (0.13 g) was stored in dark brown bottle and was kept in refrigerator. This process was repeated six times to get sufficient amount of oil to perform antibacterial assay. Yield of essential oil obtained having sharp essence was 0.22%.

Test Microorganisms

Stock cultures of microbial strains viz: *Pseudomonas aeruginosa, Escherchia coli, Klebsiella* sp.*, Proteus mirabulus,* and *Staphylococcus aureus* were collected from Pathology Lab, Jinnah Hospital, Lahore, Pakistan.

Antibacterial assay

The agar diffusion method was used for antibacterial assay (Murray *et al*., 1995). Petri plates were prepared by pouring 20 ml of LB medium and allowed to solidify. Plates were solidified and 1 ml of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained away and the inoculum was allowed to dry for 5 min. Then a Whatman No. 1 sterile filter paper disk (6 mm diameter) was impregnated with 5 µl and 10 µl essential oil, using a capillary micro-pipette. Standard reference antibiotic tetracycline (10 µg $disc^{-1}$) was used as controls for the tested bacteria. The plates were incubated at 37 °C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition using a vernier caliper against the tested bacteria. Each treatment was replicated three times.

Statistical analysis

Data were analyzed by analysis of variance followed by Dunken's Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

The *O. sanctum* essential oil displayed marked antibacterial efficacy against all the bacteria tested (Table 1). *O. sanctum* oil exhibited maximum inhibitory effect against *S. aureus*, and marked antibacterial efficacy against nosocomial pathogens, *P. mirabulus, P. aeruginosa, Klebsiella* sp. and *E. coli* (Table 1). In the present studies, bactericidal action of essential oil was found to be significantly enhanced with increase in concentration of essential oil from 5 to 10 µl against all the test microorganisms.

In a similar study, Cock (2008) reported the antimicrobial activity of *O.sanctum* leaves against bacteria and yeast. The diameter of inhibition zone recorded in *E.coli* was 18 mm for 22 µl of oil. Present investigation, in contrast showed considerable antibacterial activity 17.8 mm zone of inhibition (Table 1) even at lower concentration 10 µl. of oil. These differences may be attributed due to presence of antibacterial component in high concentration in local variety enhancing the medicinal importance of indigenous essential oil. Sattar and Shahid (1972) studied the composition of Pakistani Tulasi leaves and indicated that the essential oil is an important one as it has a sweet fragrance, high % of eugenol (61.2%) considered as antibacterial component (Mondal and. Mahapatra., 2007), and other phenolic substances e.g. methyl eugenol (1.8%), carvacrol (30.4%) and the oil has a potential of becoming a commercial commodity.

Essential oils possess antimicrobial properties (Misra *et al*., 1978) and is supposed due to the presence of monoterpene components mostly phenolic in nature which exert membrane-damaging effects to microbial strains and stimulates leakage of cellular potassium ions which is responsible for a lethal action related to cytoplasmic membrane damage. Herbal medications in the form of essential oils have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to natural preparations as compared to synthetic pharmaceuticals. With the reduced costs of essential oils preparation, makes the search for natural therapeutics an attractive option.

Table 1: Antibacterial activity of essential oil of *Ocimum sanctum* L.

Sr. No	Bacterial strains	Zones of inhibition		
		Tetracyclin (10 µg/disc)(mm)	Volume of oil used (5 µl)(mm)	Volume of oil used (10 µl)(mm)
1.	*Escherchia coli*	20b	11.5d	15.4c
2.	*Klebsiella* sp.	20b	15.1d	20b
3.	*Proteus mirabulus*	20b	10.2d	20b
4.	*Pseudomonas aeruginosa*	20b	10.2d	17.8b
5.	*Staphylococcus aureus*	20b	20b	41.5a

For each value followed by the different letter in the columns are significantly different ($P \leq 0.05$) according to DMR test.

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