

Full Length Research Paper

Antibacterial evaluation and phytochemical screening of Methanolic Extract of *Ocimum sanctum* against some common microbial pathogens

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Whole plant methanolic extract of *Ocimum sanctum* was studied for their phytochemical and antibacterial activities. Antimicrobial activity of extract was detected against some common microbial pathogens. Methanolic extract of *Ocimum sanctum* exhibited antibacterial activity against *Enterococcus faecalis*, *Enterobacter cloacae*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*. Methanolic extract of *Ocimum sanctum* showed strong antibacterial activity against *Staphylococcus aureus* and *Staphylococcus saprophyticus*. The minimal inhibitory concentrations for all tested strains were at concentrations ranging from 10.19±0.08mg/ml to 32.02±1.98 mg/ml. Phytochemical analysis of active extracts demonstrated the presence of common phytoconstituents like tannins, glycosides, saponins, flavonoids and alkaloids. These phytochemical established a good support to the use of this plant in herbal medicine and as a base for the development of new drugs, phytomedicine and might be aid in the treatment of UTI.

Keywords: Antimicrobial, indigenous medicine, Agar diffusion, UTI.

INTRODUCTION

Natural products are important sources for biologically active drugs (Saleh et al., 2009). There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world (Gazzaneo et al., 2005). According to World Health Organization

(WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of

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great importance (Diallo et al., 1999). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not adequately evaluated (Balandrin et al., 1985). The medicinal value of these plants depends on bioactive phytochemical constituents that

Produce definite physiological action in the human body. Some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils, tannins and saponins (Krishnaiah et al., 2009). Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Photochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah et al., 2007).

Ocimum sanctum commonly known as holy basil (English), or Tulasi (local language), is a herbaceous sacred plant found throughout India. Indian material medica describes the use the plant in a verity of ailments. In Indian mythology the plant is considered to extirpate all sins and purify the body when touched. It is often grown outside dwellings and worshipped daily. It is said to daunt Yama, the god of death, but has a close affinity with Lord Krishna who is reputed to have grown the herb. If offered to Lord Krishna it is said to have mystical powers of protection from death, disease and misfortune (Williamson 2002). Different parts of plant like stem, flower, seed, leaves, root etc are known to possess therapeutic potential and have been used, by traditional medicinal practitioners, as analgesic (Khanna and Bhatia 2003), antibacterial (Singh et al. 2005), anticancer (Karthikeyan et al., 1999), and hypoglycemic agent (Rai et al., 1997), etc.. early studies have been reported its Wound-healing activity (Shetty et al., 2006). Anti-fertility activity (Ahmed et al., 2002). Anti-hyperlipidaemic activity (Gupta et al., 2006), Anti-inflammatory activity (Singh et al., 1996), Anti-oxidant activity (Samjon et al., 2007), Anti-stress activity (Sood et al., 2006). Anti-toxic effect (Sharma et al., 2002), Effect on

reproductive system (Kantak and Gogate 1992). Anti-thyroid activity (Panda and Kar 1998), and Cardiovascular activity (Sood et al., 2005). etc.

Earlier studies reported the various parts (leaf, fixed oil, stem) of *Ocimum sanctum* extracts biological activities, but very few literatures are available on antibacterial activity of whole plant extract of *Ocimum sanctum*. The aim of the present work is to the screening of phytochemicals and antibacterial activity of whole plant extracts of *Ocimum sanctum* against *Enterococcus faecalis*, *Enterobacter cloacae*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* microorganisms.

Plant collection

Fresh plants or plant parts were collected randomly from Gorakhpur Uttar Pradesh, India. The plant material was authenticated by Botanists Dr. Shraddha Sharma and Mrs. Nitisha Malik, Department of Biotechnology, Institute of Applied Medicines and Research, Duhai, Ghaziabad, India. Fresh plant material was washed under running tap water, air dried, homogenized to fine powder, and stored in tightened light-protected containers.

Solvent extraction

Ten grams of air dried powder was placed in 100 ml of organic solvent (90% methanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 180-200 rpm for 24 h. After 24 h, it was filtered through 4 layers of muslin cloth and centrifuged at 5000 x g for 10 min. The supernatant was collected and the solvent was evaporated. The crude extract diluted with 5% of DMSO to make the final volume one-tenth of the original volume and stored at 4 °C in air tight bottles for further studies.

Preparation of Inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring loopful bacterial cells from the stock cultures to Erlenmeyer flask of nutrient broth that were incubated with agitation for 24 hrs at 37°C. Bacterial density was adjusted according to 0.5 McFarland turbidity standards (10^8 cfu/ml).

Test microorganisms

The microbial strains investigated are identified strains and were obtained from Microbiology department, Institute of Applied Medicines and Research, Ghaziabad, all were American type culture collection, USA. The bacterial strains studied are *Staphylococcus aureus* (ATCC 25923), *Staphylococcus saprophyticus*(ATCC 35552), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 15380), *Enterococcus faecalis* (ATCC 10741),, *Enterobacter*

Table 1: Antibacterial activity of methanolic extract of *Ocimum sanctum* against test microorganisms

Microorganism	Zone of Inhibition (mm)				
	Cef	Str	Amp	Plant extract	Control
<i>Escherichia coli</i>	39±1.82	43±2.11	-	13±0.32	0.0
<i>Enterobacter cloacae</i>	-	-	-	18±0.11	0.0
<i>Enterococcus faecalis</i>	-	27±0.76	-	13±0.09	0.0
<i>Proteus vulgaris</i>	15±0.66	29±0.68	-	12±0.03	0.0
<i>Klebsiella pneumoniae</i>	-	29±0.54	-	12±0.05	0.0
<i>Staphylococcus aureus</i>	25±1.02	31±1.98	33±1.42	20±1.32	0.0
<i>Staphylococcus saprophyticus</i>	39±1.76	45±2.08	43±2.21	20±1.51	0.0

Cef- Cefotaxime, Str- Streptomycin, Amp- Ampicillin

cloacae(ATCC 10699), and *Proteus vulgaris* (ATCC 12454). Microorganisms were maintained at 4 °C on nutrient agar slants.

Photochemical analysis

The photochemical components of the *Ocimum sanctum* plant extracts were screened by using standard procedures (Sofowora 1982; Brindha et al., 1977; Trease and Evans 1989).

Antimicrobial susceptibility test

The disc diffusion method (Bauer et al., 1966), was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum (0.5 McFarland standard) suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. 50 µl concentration of test extract was loaded on 0.5cm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. For each bacterial strain, negative controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter and the result obtained was tabulated. For positive control, three antibiotics, namely Cefotaxime (30 mcg/disc), streptomycin (10 mcg/disc) and Ampicillin (10 mcg/disc) were used. These studies were performed in triplicate.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) was determined using micro-broth dilution methods [28]. Concentrations of extract (1-9 mgmL⁻¹) dilutions were prepared using tubes containing 9 ml of double strength broth. The tubes were inoculated with the suspension of standardized inocula (0.5 McFarland standard) and incubated at 37°C for 24 h. MICs were recorded as the lowest concentration of extract showing no visible growth of the broth.

RESULTS

The results of phytochemical screening revealed that the methanol extract of *Ocimum sanctum* positive for Alkaloids, flavonoids, terpenes, saponins, glycosides , Rasins and tannins where as negative for Phenols (Table 3). The results for antibacterial activity screening of the methanolic extract of *Ocimum sanctum* are shown in Table

1. The plant extract showed antibacterial activity against all test microorganisms. The most potent effect, related to methanol extract of *Ocimum sanctum* showed inhibition effect against *Staph. aureus* as well as *Staph. saprophyticus* in similar degree of inhibition zone diameter (20±1.32 & 20±1.51 mm). MIC values for the active extract are indicated in Table 2. As shown in table 2 methanol extract of *Ocimum sanctum* with the MIC value of 10.19±0.08 mg/ml revealed that *Staph. saprophyticus* was the most susceptible bacterium which confirmed the results of disc diffusion method.

Table 2: Minimal inhibitory concentration (MIC) of *Ocimum sanctum* methanolic extract

Microorganism	MIC mg/mL
<i>Escherichia coli</i>	32.02±1.98
<i>Enterobacter cloacae</i>	25.87±1.02
<i>Enterococcus faecalis</i>	18.68±0.76
<i>Proteus vulgaris</i>	32.02±1.76
<i>Klebsiella pneumoniae</i>	25.87±1.23
<i>Staphylococcus aureus</i>	18.68±0.95
<i>Staphylococcus saprophyticus</i>	10.19±0.08

Table 3: Phytochemical analysis of methanolic extract of *Ocimum sanctum*

S. No.	Phytochemical	Methanolic Extract
1	Phenols	-
2	Alkaloids	+
3	Flavonoids	+
4	Saponins	+
5	Tannins	+
6	Glycosides	+
7	Rasins	+
8	Terpenes	+

DISCUSSION

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak.

In this study Phytochemical analysis of active extract demonstrated the presence of common phytoconstituents like tannins, glycosides, saponins, flavonoids and alkaloids. These are believed to be responsible for the observed antibacterial effects. Some studies have also attributed to their observed antimicrobial effect of plant extracts to the presence of these secondary plant metabolites (Nweze et al., 2004). The presence of tannins suggests the ability of

this plant to play a major role as antidiarrhoeic and antihemorrhagic agent (Price et al., 1987). Presence of saponins revealed immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Asquith and Butler 1986). The presence of cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure (Sood et al., 2005). This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension. The presence of these photochemical bases in *Ocimum sanctum* accounts for its usefulness as a medicinal plant.

According to the antibacterial assay done for screening purpose, all these selected gram positive and gram negative microorganisms showed zone of inhibition against test plant extract. Among these test microorganisms *Staphylococcus aureus*, and *Staphylococcus saprophyticus* are the most susceptible to methanol extract of *Ocimum sanctum*. *Staphylococcus aureus* had great medical significance in causing common problems of bacterial infections found in the community (Thamlikitkul et al., 2001). Duguid et al. reported *S. aureus*

as the causative agent of wide variety of disease of suppurative infections such as boils and, wound infections, superficial infection such as skin pustule, subcutaneous and sub-mucosa abscesses, osteomyelitis, bronchopneumonia and food poisoning, a common cause of vomiting and diarrhea. It is the commonest cause of infection in hospitals and is most liable to infect newborn babies, surgical patients, old and malnourished persons, and patients with diabetes and other chronic diseases (Duguid et al., 1978). It is also the leading cause of urinary tract infection (UTI) (Akerelle and Ahonkhai 2000; Abdul and Online 2001).

Our Current study revealed that the minimum inhibitor concentration (MIC) of *Staphylococcus saprophyticus* ($10.19 \pm 0.08 \text{ mg/mL}$) is lesser than the other test microorganisms and which was followed by *S. aureus* ($18.68 \pm 0.95 \text{ mg/mL}$). *Staph. saprophyticus* is an uropathogenic staphylococcus frequently isolated from young female outpatients with uncomplicated urinary tract infections. *S. saprophyticus* is a true urinary tract pathogen causing both upper and lower urinary tract infections (Latham et al., 1983).

Whole plant methanol extract of *Ocimum sanctum* showed lower zone of inhibition to *Proteus vulgaris* and *Klebsiella pneumonia*. Interestingly the zone of inhibition of test plant extract against *Proteus vulgaris* is was almost near to the zone of inhibition Cefotaxime. The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. An important characteristic of plant extracts 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 (Rastogi and Mehrotra 2002).

These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. In conclusion, the results of the present study support the folkloric usage of the *Ocimum*. This antibacterial study of the plant extract demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use this plant in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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