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Antimicrobial activity of leaf extract of *Ocimum sanctum* on various clinical isolates

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Abstract:

Medicinal properties are present in different parts of *Ocimum* sps. like leaves flowers, root, stem etc. and have been used by traditional medical practitioners. The aim of this study was to determine the antimicrobial activity of cold and hot water extract of leaves of *Ocimum sanctum* against clinical bacterial isolates by disc diffusion method. Cold and hot water extract of leaf of *Ocimum* sanctum were prepared at 250µg/ml, 500µg/ml, 1000µg/ml concentrations, impregnated into discs of 6mm diameter and tested on *S.aureus*, *B.subtilis*, *E.coli*, *Proteus mirabilis*, *Shigella dysentriae*, *Salmonella typhimurium* . There was no significant difference between the zone of inhibition of the test organisms to cold and hot water extracts. *S. aureus* and *S. typhimurium* had the zone size of 1.2 cm, 1.1 cm and 1.0 cm zone of inhibition for *B. subtilis* and *Shigella dysentriae* respectively. The inhibition was the greatest at concentration of 1000 µg/ml for both cold and hot water extracts against all test organisms. The antimicrobial activity was the maximum for *E. coli* i.e. 1.4 cm in both cold and hot water extracts. The antimicrobial susceptibility pattern of leaf extract of *Ocimum sanctum* was found to be more or less active against all tested pathogenic strains for both cold and hot water extracts; it may be due to the presence of tannins, essential oils, flavonoids, alkaloids and eugenol present in varying proportions. The plant contains important bio-active compounds and hence has found use as essential plant species in traditional medicine for treatment of various diseases.

Key words: Antimicrobial activity, extracts, *Ocimum sanctum*, zone of inhibition

Introduction:

The burden of infectious diseases is very high worldwide and deaths due to these are on the rise.[1] The antibiotic era to treat infectious diseases started in 1950s, which reduced the use of plant antimicrobials drastically. The traditional healing systems which rely on the medicines from natural sources took a backbench. The increase in emergence and spread of antibiotic resistance among microorganisms have initiated the scientists to look for new antimicrobials of natural or synthetic origin.[2]

Pharmacology industry has produced a number of new antibiotics, but bacteria have genetic ability to transmit and acquire resistance to these drugs too and make them less effective as therapeutic agents. Apart from microbes, plants also serve as an important source of antimicrobial agents. They produce a large number of secondary metabolites some of which have a great potential as antimicrobial agents and can have significant activity against human pathogens. [2]

About 80% of population in developed countries use traditional medicines which are compounds from medicinal plants. The use of plant products in various ailments has been advocated because they are safe, economical, effective and easily available.[1]

Ocimum sanctum (Tulsi) belonging to the family Labiatae is an aromatic herb or under shrub used in Ayurvedic treatment. Several studies have been carried out by Indian scientists and researchers to establish the therapeutic use of this perineal herb. Several medicinal properties have been attributed to different parts of

Ocimum sps. like leaves, flowers, root, stem and have been used by traditional medical practitioners as expectorant, analgesic, anti-cancer, anti-diabetic, anti-fertility, hepatoprotective, anti-asthmatic, anti-emetic, hypotensive and anti-stress agent.[3]

The aim of this study was to determine the antimicrobial activity of cold and hot water extract of leaves of *Ocimum sanctum* against a few gram positive and gram negative bacterial isolates.

Materials and Methods:

Collection and identification of plant material:

Fresh leaves of *Ocimum sanctum* were collected randomly from Namachelumae herbal garden, Tumkur, India. Taxonomic identification was done at the department of botany, Sri Siddaganga Science College, Tumkur. The leaves were washed in tap water, air dried and homogenized to fine powder in an electric grinder. The extract was prepared by Alede and Irobi method (1993) with minor modification.[4]

Cold Water Extract[4]:

Five grams of shade dried *Ocimum* sanctum leaves were homogenized with 5ml of sterile distilled water (1:1w/v) and filtered through a double layered cheese cloth. The filtrate was collected and evaporated at room temperature. 5mg of the collected residue was dissolved in 5ml of 5% Dimethy Sulphoxide (DMSO) and considered as cold water extract.

Hot water Extract[4]:

5g of dried *Ocimum sanctum* leaves was homogenized in 5ml of sterile distilled water (1:1 w/v) heated at 100^{o}C and filtered through a double layered cheese cloth. The filtrate was collected and evaporated under room temperature; 5mg of the collected residue was dissolved in 5ml of 1% DMSO and considered as hot water extract.

The extracts were tested against various bacteria in concentrations of 1000µg/ml, 500 µg/ml, and 250 µg/ml by disc diffusion method.

Three different concentrations were prepared using DMSO as the solvent [3].

a) 1000 µg/ml of the plant extract= 5mg leaf residue dissolved in 5ml of 1% DMSO.

b) 500 µg/ml of the plant extract= 2.5mg leaf residue dissolved in 5ml of 1% DMSO

c) 250 µg/ml of the plant extract=1.25mg leaf residue dissolved in 5ml of 1% DMSO.

Preparation of disc[5]:

Antimicrobial assay of cold and hot water extracts of leaves of *Ocimum sanctum* were performed against test organisms by modified Kirby-Bauer disc diffusion ion method, standard strains of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were also tested. Discs of standard size (6mm) diameter was prepared using Whatman no.1 filter paper. These were sterilized in hot air oven at 160^{o}C for 1 hour. The sterile discs were impregnated with different concentrations of plant extract and allowed to dry to remove any residual solvent and stored at 4^{o}C for use. Control discs were also prepared with 1% DMSO.

Preparation of bacterial suspension:

Stock cultures were maintained at 4^{o}C on nutrient agar slants. A loopful of the organisms was inoculated onto nutrient broth was incubated for 6hrs at 37^{o}C. The test organisms were inoculated on MacConkey agar and incubated overnight at 37^{o}C. Colonies of the organism were inoculated in peptone water and incubated. The growth in the tube was calibrated to 10^{5} MacFarland turbidity. Lawn culture of the test organisms was made on nutrient agar medium and discs were placed on the media maintaining a distance of 15mm between two discs, control disc with 1% DMSO

Organisms used in the study:

Bacterial strains studied were *S.aureus*, *B.subtilis* among gram positive bacteria and *E.coli*, *Proteus mirabilis*, *Shigella dysentriae*, *Salmonella typhimurium*

among gram negative bacteria. These test organisms were clinical isolates obtained from patients diagnosed for various bacterial infections in Sri Siddhartha Medical College, Tumkur during the period June 2010; *S.aureus* ATCC 25923 and *E.coli* ATCC 25922 standard strains were also included in the study.

Results:

Medicinal plants are a source of inspiration for novel drugs and have made huge contributions to human health. The traditional healers have made use of water as a solvent for these extracts.

The results of the antimicrobial screening for effectiveness of leaf extract *Ocimum sanctum* against gram positive and gram negative bacteria by agar disc diffusion method are depicted in **Table I** & **II**.

The inhibition was the greatest at concentration of 1000 µg/ml for both cold and hot water extracts against all test organisms. The antimicrobial activity of standard strains matched that of the test organisms. The antimicrobial activity the maximum for *E. coli* i.e. 1.4 cm in both cold and hot water extracts. There was no significant difference between the zone of inhibition of the test organisms to cold and hot water extracts. *S. aureus*, *S. typhimurium* had the zone size of 1.2 cm, 1.1 cm and 1.0 cm zone of inhibition for *B. subtilis* and *Shigella dysentriae* respectively.

Discussion:

In recent years, there has been a resurgence of interest in investigation of the traditional health promoting uses of medicinal herbs due to rapidly expanding body of modern scientific information currently available on the therapeutic value of them. *Ocimum* sps has frequently been mentioned as one of the main pillars of herbal medicine. It is also called as the king of herbal medicine.[1,6,7]

The disc diffusion method of antimicrobial susceptibility testing described by Bauer was employed which is the method commonly used to check the antimicrobial activity. The pattern of growth of the organisms on the inoculated plate begins at the point distinct from the crude extract

Table I: The antimicrobial activity of cold water extract of *Ocimum sanctum*

Name of the organism	Zone of inhibition in cm			
	0 µg/ml DMSO Control disc	250 µg/ml	500 µg/ml	1000 µg/ml
S.aureus	-	0.5	0.8	1.2
B.subtilis	-	0.6	0.7	1.2
E.coli	-	0.8	1.0	1.4
P.mirabilis	-	0.7	0.7	1.2
Shigella dysentriae	-	0.7	0.9	1.0
S.typhimurium	-	0.7	0.9	1.2

Table II: The antimicrobial activity of hot water extract of *Ocimum sanctum*

Name of the organism	Zone of inhibition in cm			
	0 µg/ml DMS0 Control disc	250 µg/ml	500 µg/ml	1000 µg/ml
S.aureus	-	0.6	0.7	1.2
B.subtilis	-	0.5	0.7	1.1
E.coli	-	0.7	0.9	1.4
P.mirabilis	-	0.7	0.8	1.1
Shigella dysentriae	-	0.6	0.8	1.0
S.typhimurium	-	0.6	0.8	1.2

reservoir and proceeds inwards where the concentration of the crude extract is inhibitory for the organisms.[5] Many workers have followed the Kirby-Bauer disc diffusion method. [3,5,8]

Among bacterial pathogens, gram negative bacteria were found to more susceptible than gram positive bacteria. Similar results were obtained by various workers. [9,10]

There is difference in opinion about the antimicrobial activity of *Ocimum* extracts. There are studies which show no difference in the activity towards both gram positive and gram negative bacteria, while some show gram positive bacteria are more susceptible than gram negative bacteria .[1,11]

The antimicrobial susceptibility pattern of leaf extract of *Ocimum sanctum* was found to be more or less active against all tested pathogenic strains for both cold and hot water extracts; it may be due to the presence of tannins, essential oils, flavonoids, alkaloids and eugenol present in varying proportions. Many researchers have found better results with methanol extract than aqueous extract and chloroform extract.[1,9] Phytochemicals exert their antimicrobial activity through various mechanisms tannins by iron deprivation, hydrogen binding or non-specific interactions with vital proteins such as enzymes.[12] Alkaloids act as a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition; they are also known for their toxicity to foreign cells such as bacteria .[13]

Gram positive bacteria are susceptible to the leaf extract of *Ocimum sanctum;* the peptidoglycan layer of these bacteria is not an effective barrier and in gram negative bacteria the outer phospholipidic membrane makes the cell wall impermeable to lipophilic comounds.[14] In traditional medicine, water has been used as the main solvent primarily for drug absorption but many scientific works have proved that alcoholic extracts are much better and more powerful.[6] The antimicrobial activity of leaf extract of *Ocimum sanctum* may be due to the attachment of the extract components to the surface of cell membrane disturbing permeability and respiratory functions of the cell. The interaction of plant extract with microbial cytoplasmic components and nucleic acids can inhibit the respiratory chain enzymes and interferes.[8]

The antimicrobial activity against *E coli* was 1.4cm with both cold and hot water extract. Studies from various parts of the country have also shown results where antimicrobial activity against *E.coli*, *S. aureus* and *S.typhimurium* is high.

In the present study, the antimicrobial activity was least at concentration of 250 µg/ml and highest at 1000 µg/ml of the extract. Many studies have been conducted to show antimicrobial activity with different types of extractions like alcoholic, chloroform etc. other than cold and hot water extracts with better zones of inhibition at higher concentrations. [1,2,9,10]

The results of the study suggest that leaves of *Ocimum sanctum* and other species of O*cimum* contain pharmaceutical compounds which can be used against both gram positive and gram negative organisms.

Conclusion:

Leaves of *Ocimun sanctum* were extracted by cold and hot water method. It has been shown that leaves of this plant contain alkaloids like boldine, tannins, saponin, glycosides, sterols etc. which possesses antimicrobial properties against gram positive and gram negative bacteria. Extract from other plant parts have also been studied and found to have similar antimicrobial activity. The plant contains important bio-active compounds and hence has found use as essential plant species in traditional medicine for treatment of various diseases. Further work is needed to isolate the active principle from plant extracts to carry out pharmaceutical studies.

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