ANTIBACTERIAL ACTIVITY OF HOT AQUEOUS EXTRACT OF OCIMUM SANCTUM LEAVES AGAINST COMMON BACTERIAL PATHOGENS OF ANIMALS

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ABSTRACT

Ocimum sanctum, holy basil is well known for its antimicrobial, anti-inflammatory, anticancer and many other medicinal activities in traditional medicine system including Ayurveda of India. In traditional medicine different parts as leaves, stem, roots, seeds flowers are well narrated for medicinal activities. There are different preparations from plant and parts of plant, however hot aqueous extract preparation is most commonly used household method of extract preparation in India. On the basis of all these the present study was designed to study the antibacterial activities of hot aqueous extract of leaves of the plant against the common bacterial pathogens of human and animals viz. Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus vulgaris obtained from the different cases of illness in animals. Many of these are of zoonotic importance and produce a serious threat to human life. While performing the antibacterial activity aqueous extract of Ocimum sanctum leaves showed dose dependent antibacterial activity. Moreover, the antibacterial effect varied bacteria to bacteria. The iscs containing 10 and 20 mg of extract revealed inhibitory effect against all the pathogens, thus proving the traditional use of Ocimum sanctum in common ailments in Indian traditional system of medicine Ayurveda.

Keywords: Ocimum sanctum, antibacterial activity, hot aqueous extract.

INTRODUCTION

Ocimum sanctum plants are considered as one of the most important source of medicine and drugs with many secondary metabolites and essential oils recommended for treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye disease, chronic fever, eye disease etc.[1,2]. Ocimum sanctum also posses antifertility, anticancerous, anti diabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic, and diaphoretic activities [2,4,5,6]. Various preparations of different part of Ocimum
sanctum plants have been proved to be economical and effective and on the other hand they are easily available and safe to use \cite{7,8,9,10}. Ocimum sanctum is distributed mainly in the tropical and subtropical region of the world and is also considered to be highly scared, medicinal application in the indigenous system of medicine of many Asian, African and South American countries \cite{2}. Different parts (leaves, stem, flower, root, seed and even whole plant) of Ocimum sanctum have been applied in different ailments particularly against bacterial pathogens. Bacterial organisms viz; Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus vulgaris are well known pathogens not only for human but also for animals and other living species \cite{11}. Considering all these issues most commonly used preparation of Ocimum sanctum plant hot aqueous extract, with most routinely used part leaves of plant were selected for antibacterial activities against common bacterial pathogens.

**MATERIALS AND METHODS**

**Collection of leaves of Ocimum sanctum:** Leaves of O. sanctum were collected from the campus of UP Pt. Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura and dried under shade. The biological specimen of the same was verified by Department of Botany, BSA College Mathura and preserved in Department of Microbiology. Dried leaves were used for preparation hot aqueous extract.

**Bacterial isolates:** Bacterial organisms viz; Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus vulgaris were used to study antibacterial properties of extract of Ocimum sanctum leaves. All these organisms were obtained from the Department of Microbiology and Immunology, Pt Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura. Prior to use all the bacterial isolates were characterized on the basis of morphological, cultural and biochemical characteristics \cite{12}.

**Extract preparation:** Hot aqueous extract (HAE) of O. sanctum leaves was prepared \cite{11}. In this method the 50 gram of dried powder of O. sanctum leaves was placed in a porous cellulose thimble. The thimble was then placed in an extraction chamber, above a collection flask containing the 750 ml solvent (triple glass distilled water). The flask was heated and the solvent was allowed to evaporate. Temperature was adjusted according to
boiling temperature of the solvent (100°C). The extraction process lasted 6-8 hours and then flask containing the solvent and extract were removed. The solvent in the flask was allowed to evaporate and finally the remaining material was collected and weighed.

**Bacterial concentration for in vitro activity:** McFarland’s nephelometer for determination of bacterial concentration was prepared and 0.5 ml of respective bacterial cultures containing approximately 3x10^4 cells/ml bacterial cell suspension / plate using three nutrient agar plates per experiment was used [11].

**Preparation of antibiotic discs:** The antibacterial effect was studied by disc diffusion method [11]. Discs containing four different concentration 2 mg / 5 mg /10 mg / 20 mg of *O. sanctum* hot aqueous leaves extract were used to study the antimicrobial activity and were planted at even distance (8-10 mm) on nutrient agar plates on which bacterial culture were streaked.

**Antibacterial effect of extract:** The culture plates were incubated at 37°C for 48 hours. The antimicrobial activity of extract marked by the zone of inhibition and bacterial growth around the disc was measured in mm by scale. Prior to use discs were tested for sterility. Zone of inhibition was measured at interval of 6 hours from 24 to 48 hours [1].

**RESULTS & DISCUSSION**

**TABLE 1: ANTIBACTERIAL EFFECT OF HOT AQUEOUS EXTRACT OF *O. SANCTUM* LEAVES**

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Quantity of Ext./disc (mg)</th>
<th>Zone of inhibition (mm)</th>
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<td>24 hr</td>
<td>30 hr</td>
<td>36 hr</td>
<td>42 hr</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>No zone(R)</td>
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<td>Escherichia coli</td>
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<td>Salmonella</td>
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<td>Typhimurium</td>
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<tr>
<td>Klebsiella pneumonia</td>
<td>2</td>
<td>No zone(R)</td>
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<td>Pseudomonas aeruginosa</td>
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<td>Proteus vulgaris</td>
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R- Resistant

Results shown in the table prove the antibacterial activities of O. sanctum against the various clinical isolates. The antibacterial effect is dose dependent and it increases with the increase of dose. The effect of HAE varied organism to organism as the discs containing 2 mg of HAE were not effective against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Proteus vulgaris. The discs of 5 mg HAE were not able to inhibit the growth of Escherichia coli and Pseudomonas aeruginosa however, Salmonella Typhimurium was found to be sensitive to all the concentrations of HAE applied during study. The higher concentration was able to inhibit the growth of all the pathogens under study. These variations might be due to virulent attributes of bacterial pathogens as Pseudomonas aeruginosa are considered to be very notorious bacteria for their antibiotic and antimicrobial resistant[3]. Similarly many other workers applied different preparations against common pathogens viz., E. coli and Staph. aureus[13]; Escherichia coli, Bacillus anthracis, Bacillus subtilis, Salmonella Newport, Salmonella pullorum, Staphylococcus aureus, P. vulgaris and Pseudomonas aeruginosa[14]; Mycobacterium tuberculosis, Arthrobacter globiformis, B. megatherium, E. coli and Pseudomonas[15,16,17,18]; E. coli, Klebsiella aerogens, Proteus vulg.
mirabilis, Salmonella typhimurium, Shigella dysentriae, Pseudomonas aeruginosa, Vibrio cholerae and Staphylococcus aureus \cite{19}; Pasturella multocida, E. coli, Bacillus subtilis and Staphlococcus aureus \cite{20}; Salmonella typhi, Salmonella paratyphi A and Salmonella typhimurium \cite{21} and E. coli, Klebsiella spp., Bacillus spp., Staphylococcus aureus, Pseudomonas aeruginosa \cite{11} and reported variable effectiveness. However, with slight variations all these findings support the traditional use of Ocimum sanctum parts to cure and prevent human and animal ailments of bacterial origin. Thus it can be concluded that hot aqueous extract of O. sanctum leaves has antibacterial activities justifies its traditional use as antibacterial agent.

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