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Antimicrobial activity of *Ocimum sanctum* leaves extracts and oil

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ABSTRACT

The aim of this study was to evaluate the antimicrobial effects of ethanol, Hexane and Chloroform leaf extracts of O. sanctum against pathogenic bacteria to determine their potentials as antibacterial agent. *Ocimum sanctum* Linn known as Holy Basil is an aromatic plant native to the tropics of Asia and Africa being medicinally important plant in the family Lamiaceae. The family Lamiaceae is one of the most exploited medicinal plant family worldwide not only as a source of medicinal plants but also with its valuable essential oils being used as spices and flavours for various food products. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed. Leaves were separated from the stem, washed in clear water and dried until they were adequately dry to be ground (dried for 7 days). Dried leaves were powdered separately in an electric grinder until a homogenous powder was obtained. Ethanolic, Hexane, Chloroform extract was prepared from the powder obtained using "cold extraction method. The activity of Tulsi extract against K. pneumonia & *Staphylococcus aureus* was found to be higher at a concentration of 100% followed by 75% & 50% respectively. The maximum zone of inhibition was found to be 21&11mm against *Kleibsiella pneumonia* and 18&15mm against *Staphylococcus aureus*. The antimicrobial efficacy of *Ocimum sanctum* leaves indicates that the plant possesses potent antimicrobial properties as well as *Ocimum* is widespread in India, it can be recommended as an easily available and renewal source of antimicrobial agent instead of synthetic chemicals.

Keywords: *Ocimum santum, Staphylococcus aureus, Klebsialla pneumonia,* Disc Diffusion

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INTRODUCTION

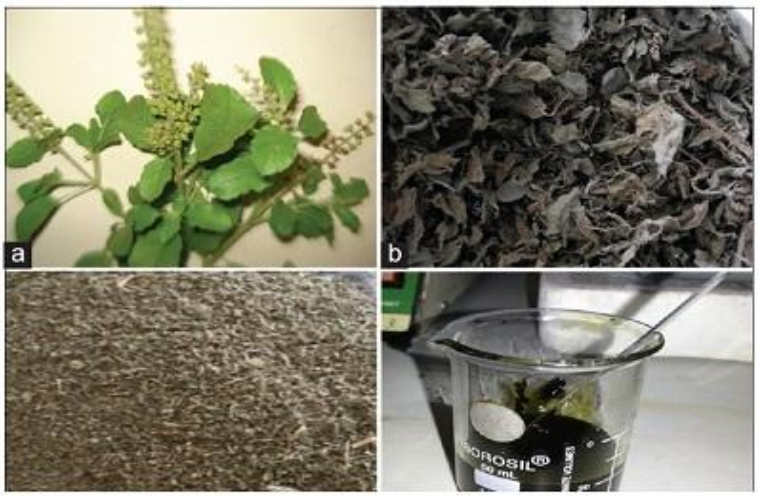
Ocimum sanctum Linn known as Holy Basil is an aromatic plant native to the tropics of Asia and Africa being medicinally important plant in the family Lamiaceae. The family Lamiaceae is one of the most exploited medicinal plant family worldwide not only as a source of medicinal plants but also with its valuable essential oils being used as spices and flavours for various food products. Morphologically O. sanctum is an erect about 75 cm tall, much branched with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed [8,9,10].

The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against currently available antibiotics. However, traditional plants have been proved to be better source for novel antimicrobial drugs. Most of Indian plants accounts for the richest resources of natural drugs [4]. Historically medicinal plants have been placed at top among the source of novel drugs with anti-microbial activity. These traditional medicinal herbs have made considerable contributions to human health. In addition, plants are considered as one of the most important sources of secondary metabolites and essential oils [5]. On one hand the use of medicinal plants proved to be economical and effective and on the other hand they are easily available and safe to use [6]. Indian traditional medicinal system includes hundreds of medicinal plants related to multiple effects [7]. Furthermore, studies have revealed O. sanctum to possess anti-inflammatory, analgesic, antipyretic, antidiabetic, hepatoprotective, hypolipidemic and antistress activities [11]. Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal, nematicidal and fungistatic. The disc diffusion method is the commonly used technique to check the antimicrobial activity. The antimicrobial activity was tested by the method based on the principle of Kirby Bauer test. An antimicrobial is a

substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, all protozoans (Merriam-Webster Online Dictionary) [12]. The aim of this study was to evaluate the antimicrobial effects of ethanol, Hexane and Chloroform leaf extracts of O. sanctum against pathogenic bacteria to determine their potentials as antibacterial agent.

MATERIAL AND METHODS

The study employed an *in vitro* experimental design. Tulsi leaves were obtained from courtyards and local market from Shivalik hills of Himachal area. Authenticity of plant by Dr GS Chatha, Shivalik College, Department of Botany, Nangal. Leaves were separated from the stem, washed in clear water and dried until they were adequately dry to be ground (dried for 7 days). Dried leaves were powdered separately in an electric grinder until a homogenous powder was obtained. Ethanolic, Hexane, Chloroform extract was prepared from the powder obtained using "cold extraction method [1]. Three hundred grams of finely powdered *Ocimum sanctum* (Linn.) was then macerated with *Tulsi (Ocimum santum) extract preparation; (a) Ocimum sanctum plant; (b) leaves separated and dried; (c) leaves ground to powder; (d) extract obtained* 100% ethanol and other solvent. It was then subjected to filtration with Whatman filter paper to obtain a clear filtrate. The filtrate so obtained was reduced at a low temperature of less than 60 ^{o}C to obtain a solid residue of *Ocimum sanctum* (Linn.) extract[2]. From 300 grams of *Ocimum sanctum* (Linn.) powder dissolved in 1 liter of ethanol and other solvent, 18 gram of extract (residue) was obtained and thus the yield was 6% w/v. Accurately weigh 1gm of each extract was reconstituted in 10 ml of respective solvents to obtain stoke solution in eppendorf tube. Further the dilutions were made with respective solvents. Accurately weigh of 10 mg of Standard Gentamycin was dissolved in 10 ml of distilled water to get 1mg/ml. The different dilutions and standard pipetted out on into the marked plates. These were left for incubation at 37°C for 24 hrs and 25º C for 36 hrs. After incubation zones of inhibition were measured (from antibiotic zone measurement scale) in mm and compared with standard.



Collection of test organism

Collection of test organism and preparation of stock culture: The following strains were obtained for the antimicrobial tests. Gram negative bacteria were Klebsiella pneumoniae and Gram positive bacteria were Staphylococcus aureus. All the microbial strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. Nutrient broth medium was prepared and autoclaved. After the bacterial cultures were inoculated to separate flasks and incubated in shaker for 24 hours

Microbiological Assay

The test organisms included for study were gram positive Staphylococcus aureus and gram negative Klebsialla pneumonia [3].

Preparation of Media

For 100 ml of media,40 gm of muller-hinton agar is dissolved in 100 ml distilled water. 250 ml media prepared and autoclaved at 121°C to 15-20 min at 15 lbs/inch^2.

Preparation of Disk

Freshly prepared and sterilized molten media was poured onto Petri plates inside Laminar and after pouring UV light turned on to avoid contamination on plates while media solidifying. It was left for half an hour for proper solidification.

After media gets solidified than UV light is turned off and 10µl of bacterial suspension pipetted into plates and swabbed. Sterile discs were put (with the help of forceps) on plates along with one disc of standard (6 disk was put on a plate).

RESULT

The result of antibacterial activity of *Ocimum sanctum* against *Staphylococcus aureus*, and *Klebsialla pneumonia* with different concentration. The least zones of inhibition were displayed by the negative control and Gentamycin exhibited the widest zones of inhibition against all the bacteria. Tulsi leaves' extract showed increasing zones of inhibition. Results of the study were shown in the table 1. We observed that O. sanctum have a potent antibacterial activity. The ethanolic extract showed maximum antibacterial activity followed by hexane and pure oil extracts against S. aureus and K. pneumonia. etc. (Table 1 and 2). The activity of Tulsi extract against K. pneumonia & Staphylococcus aureus was found to be higher at a concentration of 100% followed by 75% & 50% respectively. The maximum zone of inhibition was found to be 21&11mm against *Kleibsiella pneumonia* and 18&15mm against *Staphylococcus aureus*.

Table 1: Tulsi leaves serial dilution showing the Zone of inhibition (in mm) against *Kleibsiella pneumonia* (Gram negative bacteria)

	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5	Dilution 6	Gentamycin
Chloroform extract	15	12	11	9	7	7	17
Haxane Extract	6	4	4	3	3	NZ	17
Alcoholic Extract	18	16	14	14	13	10	17
Pure oil	7	7	6	5	5	4	17

Table 2: Tulsi leaves serial dilution showing the Zone of inhibition (in mm) against *Staphyiococcus aureus*(Gram positive bacteria)

	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5	Dilution 6	Gentamycin
Chloroform extract	11	11	10	9	5	4	17
Haxen Extract	9	7	7	4	3	2	17
Alcoholic Extract	21	19	18	16	14	10	17
Pure oil	8	7	7	3	2	NZ	17

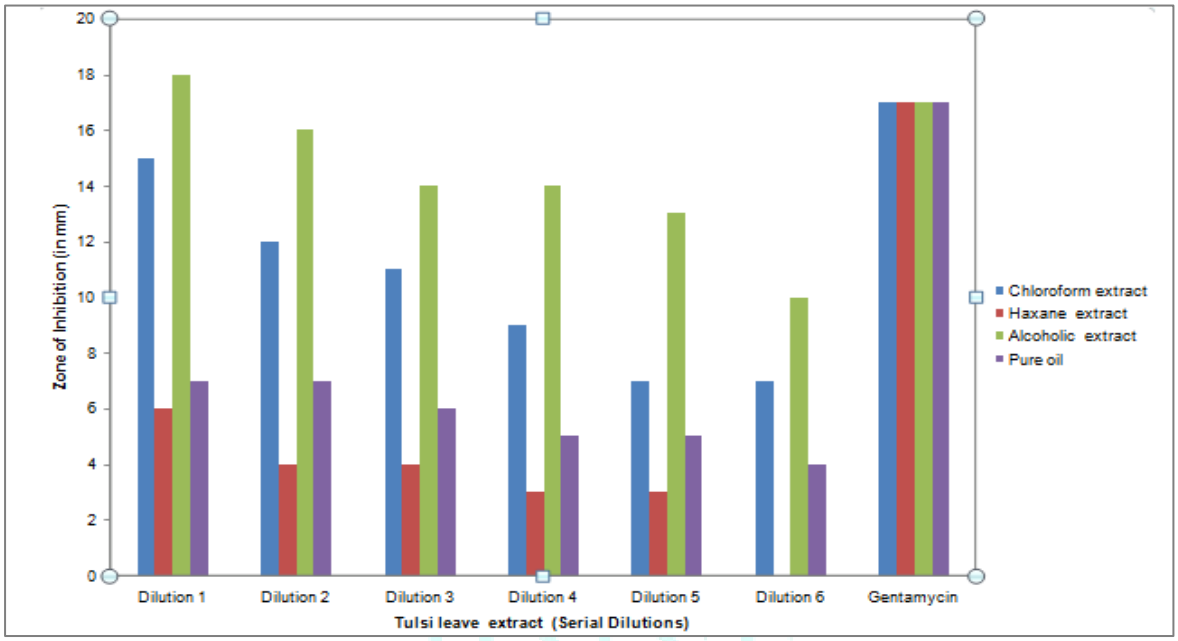


Figure 1: Tulsi leaves serial dilution showing the Zone of inhibition (in mm) against *Kleibsiella pneumonia* (Gram negative bacteria).

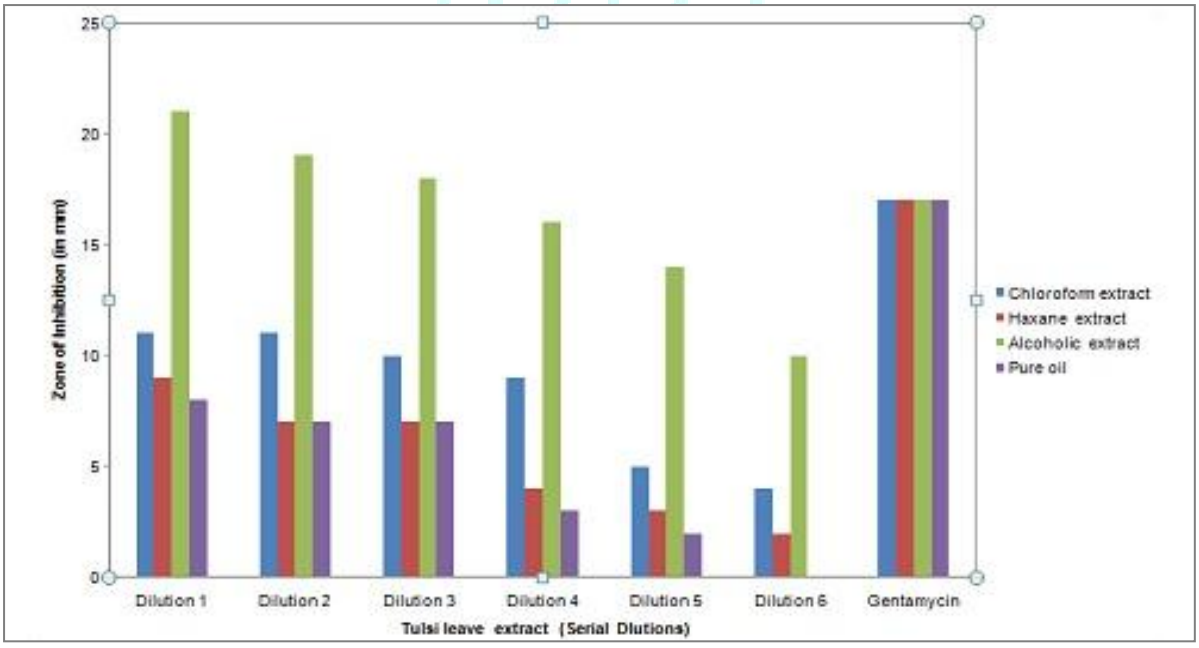


Figure 2: Tulsi leaves serial dilution showing the Zone of inhibition (in mm) against *Staphyiococcus aureus* (Gram positive bacteria).

DISCUSSION

Antibacterial activity of different *Ocimum sanctum* extracts against *Kleibsiella pneumonia* (Gram positive becteria) and Staphylococcus aureus (Gram negative becteria) were studied. According to the results, all different types of extracts obtained from *Ocimum sanctum* leaves shown to be with antibacterial activity against tested microbial pathogens. *Ocimum* extract has found to be with antimicrobial properties against *Staphylococcus aureus* and *Kleibsiella pneumonia*. Highest antibacterial activity was shown by ethanol and chloroform extracts against both gram-negative bacteria (Staphylococcus aureus) and gram-positive bacteria (*Kleibsiella pneumonia*). Chloroform extract shown higher antibacterial activity against gram negative bacteria compared to gram positive bacteria and ethanolic extract shows higher activity against gram positive bacteria compared to gram negative bacteria in the present study. The activity of Tulsi extract against *Kleibsiella pneumonia* & *Staphylococcus aureus* was found to be higher at a concentration of 100% followed by 75% & 50% respectively. As the concentration of extract increases, the activity also increases and thus the zone of inhibition too increases. Significant results were shown by Kleibsiella pneumonia and Staphylococcus aureus.

CONCLUSION

The present study clearly indicates that *Ocimum sanctum* is a rich source of phyto-chemical constituents. The antimicrobial efficacy of *Ocimum sanctum* leaves indicates that the plant possesses potent antimicrobial properties as well as *Ocimum* is widespread in India, it can be recommended as an easily available and renewal source of antimicrobial agent instead of synthetic chemicals.

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