

Potential of *Ocimum sanctum* to Inhibit the Growth of *Pseudomonas aeruginosa*, a Disease-causing micro-organism

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ABSTRACT

Aim and Objectives: Plants are a good source of secondary metabolites, which have huge potential to kill micro-organisms. The present study aimed to evaluate the antimicrobial activity of *Ocimum sanctum* ethanol extract against drug-resistant and drug-sensitive strains of *Pseudomonas aeruginosa* (*P. aeruginosa*). **Materials and Methods:** A total of 119 strains of *P. aeruginosa*, 92 multidrug-resistant (MDR) and 27 pan sensitive strains were included in the study, which were isolated from different human clinical samples. *Ocimum sanctum* crude leaves extract was prepared with the standard "cold extraction method". The susceptibility agar dilution plates were made by mixing molten Mueller-Hinton Agar (MHA) media with plant extract in different quantities to get the final extract concentration of 0.2%, 0.1% and 0.05%. These plates were used for determining the sensitive/ resistance nature of all strains of *P. aeruginosa* against plant extract. **Results:** Among 92 MDR strains in MHA plates with 0.05% of plant extract, 59 strains were sensitive and 33 were resistant. The same MDR strains in MHA plate with 0.1% of the extract showed somewhat similar trends, 61 sensitive and 31 resistant. Whereas with 0.2% of the extract, 67 strains were sensitive, 11 showed resistance while 14 showed discrete colonies. Among 27 pan sensitive strains at 0.05% concentration, there was complete growth inhibition in 23, while discrete colonies were observed in 4, though, at 0.1% and 0.2%, all strains were found as sensitive. **Conclusion:** Analysis of the present study showed that *Ocimum sanctum* extract possess compounds with antimicrobial properties against commonly used isolates of *Pseudomonas aeruginosa* strains.

Keywords: Antimicrobial activity, *Ocimum sanctum*, Tulsi, *Pseudomonas aeruginosa*, Plant extract, Drug discovery.

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INTRODUCTION

Ocimum sanctum (Tulsi) is a traditionally used medicinal plant belonging to family Lamiaceae.¹ It is native to the tropics of Asia and Africa and has a wide range of antimicrobial properties, which makes it a promising medicinal candidate.²⁻⁴ The beneficial effects of plants are primarily due to the presence of secondary metabolites and essential oils which have huge potential to kill different types of micro-organisms.⁵

The increasing global burden of drug resistant strains of diverse pathogens particularly multidrug-resistant (MDR) strains, necessitates the development of novel antimicrobial agents. Traditional medicinal plants, on the other hand, have been shown to be a good source of novel antimicrobial compounds. Furthermore, many studies have revealed that *Ocimum sanctum* (*O. sanctum*) has antibacterial,



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antifungal, antiviral, antidiabetic, anti-inflammatory, analgesic, antipyretic, hepatoprotective and anti-stress properties.^{2,6-7}

Pseudomonas aeruginosa (*P. aeruginosa*), a gram-negative bacterium is an opportunistic human pathogen implicating in urinary tract infections, respiratory infections, gastrointestinal infections and bacteraemia in patients with immunocompromised (such as cancer, HIV and others) and these infections frequently cause significant morbidity and mortality.⁸ It is easy for it to develop resistance to a variety of conventional antipseudomonal antibiotics by means of different mechanisms,⁹ and the frequency of MDR strains is on the rise.¹⁰ Quinolones bind to the A subunit of enzyme DNA gyrase that preserves the ordered structure of chromosome inside the cells. The aminoglycosides bind to the 30S subunit of ribosome, and therefore inhibit protein synthesis. The β -lactams inhibit the peptidoglycan assembling transpeptidases of the outer face of cytoplasmic membrane. Leaf extract of *O. sanctum* was found to have antimicrobial activity against various micro-organism¹¹⁻¹³ but it was not well studied against *P. aeruginosa*. Hence, considering the wide potential of *O. sanctum* plant as the source of antimicrobial agents, the present study was aimed to investigate the potential antimicrobial effect of ethanol extract of *O. sanctum* leaves against *P. aeruginosa*.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases (NITRD), New Delhi, India, from October 2019 to November 2020. This is a laboratory-based study where patients/participants were not directly contacted and hence, ethical approval was not required. Multidrug-resistant (MDR) and pan sensitive strains of *P. aeruginosa* isolated from different clinical samples were used for antimicrobial activity testing of ethanol extract of *O. sanctum* leaves.

Collection of Plant Materials and Preparation of Plant Extract

The crude leaf extract of *O. sanctum* Linn was obtained from the Department of Zoology, Deshbandhu College, University of Delhi, Delhi, India. It was prepared by cold extraction method as per the standard protocols.^{14,15} Briefly, collected leaves of plants were washed thoroughly with tap water and then with distilled water. Then they were dried at room temperature under the electric fan and powdered, which was kept at -20°C in an airtight bottle and was used for extraction. For extraction, a total of 20 gm powder was mixed with

200 ml ethanol and kept for 72 hr at room temperature. After every 4-5 hr, a stirring of the solution was done in orbital shaker. It was then filtered by utilizing Whatman filter paper number-1 to get clear filtrate. Later, it was dried to evaporate the ethanol completely under reduced pressure in Rotary Vacuum Evaporator (Buchi Type). The dried extract was measured in grams and was stored in a freezer at 4°C . The extract was diluted in the inert solvent dimethyl sulfoxide (DMSO) to get 10% stock solution (1gm per 10 ml) for further preparing plant agar dilution plates.

Isolation and Identification of Test Organism

The bacterial strains of *P. aeruginosa* were isolated from various samples, sputum (36), urine (29), pus (21), plural pus (16), endotracheal aspirates (10) and blood (7), which were obtained from Outpatient Department (OPD), Intensive Care Unit (ICU) and wards. All the strains of *P. aeruginosa* were identified by culture characteristics, Gram staining and standard biochemical tests.¹⁶⁻¹⁷ The antimicrobial susceptibility testing of *P. aeruginosa* was performed after getting culture-positive results, with *amikacin*, *gentamycin*, *cefotaxime*, *ceftazidime*, *cefixime*, *ciprofloxacin* and *tobramycin* by standard disc diffusion method.¹⁸ Based on the susceptibility report and criteria for defining MDR organism,¹⁹ *P. aeruginosa* strains were divided into 2 groups; multidrug-resistant and pan sensitive strains.

All the randomly selected *P. aeruginosa* were subjected to antimicrobial susceptibility testing (AST) with ethanolic leaf extract of *O. sanctum*. Clinical samples showing no bacterial growth or contaminated results were excluded from the study before performing AST. Standard *P. aeruginosa* strains (ATCC 2783) with known antibiotic susceptibility patterns were taken as a positive control.

Preparation of Inoculums

Colonies of micro-organisms were transferred to sterile saline and vortexed to get a smooth suspension. Turbidity was adjusted visually with 0.5 McFarland standard. This was used further for the antimicrobial test of plant extract.

Preparation of Agar Dilution Plate

Mueller-Hinton Agar (MHA) media was prepared by using 3.9 gm of MHA powder and 100 ml distilled water. It was autoclaved at 121°C at 15 lbs/inch² for 15 min. The susceptibility agar dilution media was made by mixing molten MHA media with plant extract in different concentrations to reach final extract concentrations of 0.2%, 0.1% and 0.05%. Agar dilution plates were made by pouring 15-18 ml of above-mixed media in each Petri plate (100 mm) and allowed to

harden at room temperature. The plates were stored at 4°C in the refrigerator. These were used within a week for susceptibility testing. MHA plates with only DMSO (inert solvent) without plant extract was used as negative control plate.

Antimicrobial Susceptibility Testing (AST)

All the MDR and pan sensitive strains of *P. aeruginosa* were subjected to AST. The plates containing the different concentrations of plant extract were spot inoculated with a micropipette to deliver 2 µl freshly prepared inoculum of *P. aeruginosa*. All the inoculated plates were incubated at 37°C for 48 hr. Test organism (*P. aeruginosa*) that showed growth on the control plate (without plant extract), but no growth on the agar dilution plate having plant extract was considered sensitive. If the same growth was observed on both the control and plant extract plate then it was considered resistant. Additionally, when the countable colonies were seen, it was termed as discrete growth and likely to be a mutant variant of colonies.¹⁸ Proportion statistical test was done at 5% level of significance and *p*-value was calculated.

RESULTS

A total of 119 strains of *P. aeruginosa* were selected (92 MDR and 27 pan sensitive) for the present study which was obtained from 119 patients. Among these, 78 were male and 41 were female, and their mean age was 38 (± 16, SD). These human pathogenic strains were obtained from the following clinical samples, sputum, urine, pus, plural pus, endotracheal aspirates and blood. Dose-dependent results were observed when antimicrobial susceptibility testing of leaf extract of *O. sanctum* was performed against these *P. aeruginosa* strains. Among 92 resistant strains tested with MHA plates having 0.05% of plant extract, 59 showed sensitivity and 33 were found as resistant. In the MHA plates with 0.1% extract, these strains showed somewhat similar trends, 61 sensitive and 31 resistant. However, in the MHA plates with plant extract concentration of 0.2%, 67 strains showed sensitive (*p*<0.0001), 11 were found as resistant and discrete growth was observed in the remaining 14. Of 27 pan sensitive strains of *P. aeruginosa*, 23 showed complete growth inhibition whereas the remaining 4 showed discrete colonies at 0.05%, however, no growth was observed on media plates having 0.1% and 0.2% plant extract concentration.

DISCUSSION

The antibiotic-resistant organisms have put current antimicrobial therapy in jeopardy, and so have become a

serious problem for public health strategies. Antibiotic resistance develops as a result of irrational use of antibiotic agents and it can also occur spontaneously.²⁰ Nowadays, the focus has been shifted to the search for a new antimicrobial substances from medicinal plants.²¹ The present study has been conducted to evaluate the antimicrobial activity of leaf extract of *O. sanctum* against human pathogen *P. aeruginosa* (isolated from different clinical samples like sputum, pus, endotracheal aspirate, urine, pleural pus), using MHA media with different leaf extract concentration (0.05%, 0.1% and 0.2%). The study included 92 MDR strains and 27 pan sensitive strains of *P. aeruginosa* based on antimicrobial sensitivity testing by disc diffusion method.

In the present study, plant extract of *O. sanctum* was found to have antimicrobial activity against both MDR and pan sensitive strains of *P. aeruginosa*. The maximum antimicrobial effects of the plant extract were observed at a concentration of 0.2%. The plant extract at this dose inhibited the bacterial growth completely in 67 (73%) of 92 MDR strains, hence, showing the remarkable antibacterial properties against drug-resistant strains of *P. aeruginosa*. Literature review reveals that *O. sanctum* also demonstrated antimicrobial activity against drug-resistant strains of many other types of pathogens.¹ In one such study, ethanol extract of *O. sanctum* leaves confirmed marked inhibitory impact on microorganisms *P. aeruginosa* by agar-well diffusion method.²² When 27 pan sensitive strains were tested with these plant extract concentrations, there was complete growth inhibition at 0.1% and 0.2%. Therefore, the results display that leaf extract of *O. sanctum* possesses antibacterial components towards both MDR and pan sensitive strains of *P. aeruginosa*. The present study has remarkable strength as it includes antibiotic-resistant as well as sensitive strains. Therefore, this study inferred to compare the results of antimicrobial activity of *O. sanctum* leaf extract with standard antibiotic susceptibility testing.

Antimicrobial activity of *O. sanctum* was established previously against many gram positive and gram-negative bacteria including *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Aggregatibacter actinomycetemcomitans*, indicating that this plant contains a lot of active phytochemical components showing antimicrobial activities.^{2,23-24} The previous study also noted the antimicrobial activity of *O. sanctum* leaf extract against *P. aeruginosa*,⁴ which supports the findings of the present study. However, there are only few studies available and therefore, the present study underlined the necessity for further study to explore the use of the available antimicrobial agent in *O. sanctum* against

P. aeruginosa. The antimicrobial action of phytochemicals is mediated by a variety of mechanisms. Alkaloids serve as a DNA intercalator and decrease DNA synthesis by inhibiting topoisomerase,²³ tannins' antimicrobial effect includes inhibition of oxidative phosphorylation, deprivation of substrates needed for microbial development and inhibition of extracellular enzymes.²⁵ Use of medicinal plants reveals various other benefits including the fact that they are readily available, inexpensive and safe to use.²⁶

CONCLUSION

The results of the present study suggest that *O. sanctum* extract possesses a compound with antimicrobial properties against strains of *Pseudomonas aeruginosa*, which can be used further for the drug discovery against this pathogen.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

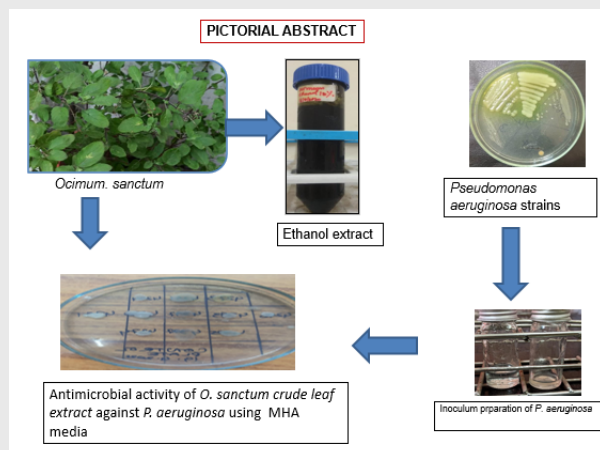
ABBREVIATIONS

MHA: Mueller Hinton Agar; **MDR:** Multidrug-resistant; **gm:** Gram; **%:** Percent; **°C:** Degree Centigrade; **DNA:** Deoxyribonucleic Acid; **SD:** Standard Deviation.

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PICTORIAL ABSTRACT



SUMMARY

The present study aimed to evaluate the antimicrobial activity of ethanolic crude leaf extract of *Ocimum sanctum* against the pathogen *Pseudomonas aeruginosa* (*P. aeruginosa*), using Mueller Hinton Agar (MHA) media. A total 119 strains of *P. aeruginosa* were included in the study of which 92 were multidrug-resistant (MDR) and 27 pan sensitive strains. Three different final concentrations of leaf extract (0.05%, 0.1% and 0.2%) were obtained with MHA media for the antimicrobial susceptibility test (AST). Dose-dependent results were observed in AST of leaf extract of *Ocimum sanctum* against *P. aeruginosa*. The maximum antimicrobial effects of the plant extract against MDR strains were observed at a concentration of 0.2% (67 sensitive, 11 resistant and discrete colonies observed in 14). Among pan sensitive strains, there was complete growth inhibition in 23 at 0.05% concentration, while discrete colonies were observed in 4, whereas all strains were found to be sensitive at 0.1% and 0.2%. Analysis of this study showed that *O. sanctum* extract possesses compound with antimicrobial properties against *P. aeruginosa*.

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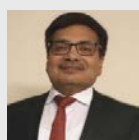
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