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Assessment of *Ocimum sanctum* Extracts as an Antifungal Agent against *Aspergillus brasiliensis*

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ABSTRACT

In many regions across the globe, there exists compelling evidence of the historical use of medicinal plants to combat diseases caused by diverse pathogenic microorganisms. Traditional therapeutic practices have relied on specific plant species known for their antimicrobial properties, which were engaged in therapeutic treatments. These plants possess a multitude of biological compounds that hold potential for the development of new drugs aimed at enhancing human well-being. A large proportion of the population in developing countries still relies on traditional folk medicine obtained from the plant resources. An attempt has been made to evaluate the antimicrobial activity of the medicinal plant- *Ocimum sanctum* based on prevalent diseases and ethnobotanical knowledge, against *Aspergillus brasiliensis*. In order to assess the antifungal efficacy of Tulsi, the agar well diffusion method was employed. A Soxhlet apparatus was used to prepare the extraction of Tulsi leaves. Five different concentrations (0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, 1.0mg/ml) of tulsi extract were prepared by employing aqueous solution as well as various solvents such as acetone, chloroform, and methanol. The extracts were subjected to microbiological investigation to evaluate antimicrobial properties of tusli. ZOI were observed in mm. At a concentration of 1.0mg/ml, chloroform displayed the maximum zone of inhibition (21mm) among the various solvents tested. The leaf extracts obtained from solvents (acetone, chloroform, and methanol) exhibited antifungal activity against *A. brasiliensis*. These findings highlight that extracts from *O. sanctum* represent valuable sources of natural bioactive compounds, with potential application as potent antimicrobial drugs for combating a range of pathogenic microorganisms.

Keywords: Antifungal activity, *O.sanctum*, Zone of inhibition, *A.brasiliensis*.

INTRODUCTION

For thousands of years, various medicinal plants have found applications in a wide range of uses, including food preservation, pharmaceuticals, alternative medicine, and natural therapies. The general consensus is that naturally produced compounds, as opposed to synthetic ones, tend to undergo more effortless biodegradation, making them more environmentally friendly and widely accepted. Consequently, natural antioxidants, antibacterial agents, cytotoxic compounds, antiviral substances, fungicidal agents, and nutrients have experienced a surge in popularity in recent times. Their utilization and favorable reputation among consumers are progressively expanding. Over the past few years, the widespread and indiscriminate use of commercial antimicrobial drugs for treating infectious diseases has led to the emergence of multidrug resistance in both human and plant pathogenic microorganisms. (Davis, 1994; Service, 1995). The search for novel therapeutic agents has led to a growing interest in plants exhibiting antimicrobial

activity (Kalemba and Kunika, 2003; Juliani and Simson, 2002; Falerio et al., 2003). The Labiatae family is widely recognized for its extensive use as a global source of spices and renowned for providing extracts with powerful antimicrobial properties. Within this botanical family, the genus *Ocimum* encompasses various species, among which *Ocimum sanctum* stands out as one of over 60 distinct Ocimum species.

Ocimum plants, their different plant parts, extracts, and essential oils, find application as both spices and flavours in a wide range of food products. Moreover, these plants have been utilized as potent remedies in folk medicine, particularly in Africa and Asia, with documented effectiveness (Sacchetti et al., 2004; Jirovetz et al., 2003). Tulsi, scientifically known as *Ocimum sanctum* or Holy Basil, is a plant widely grown worldwide and highly valued for its religious and medicinal significance, especially in tropical regions. Its medicinal properties have a strong foundation in Ayurveda, an ancient medicinal system from India, and it is acknowledged as a valuable medicinal plant in Southeast Asia. According to Ayurveda, Tulsi has been acknowledged for its therapeutic potential, including specific attributes like its antiasthmatic effects (known as Sashemani Shwasaharani) and its ability to suppress cough (referred to as Kaphaghna). Indian Herbal Pharmacopoeia, 2002; Khanna and Bhatia,2003). Over the last two decades, several Indian researchers and scientists have conducted numerous studies to emphasize the diverse benefits of Tulsi for the general public.

Fungi play a significant role in nature by decomposing organic plant materials, making them crucial for the environment, food production, pharmacy, and industry. Fungi, particularly Aspergillus, flourish on decaying fruits as their primary source of nutrition, as these fruits provide vital nutrients for their growth. The mold that develops on these fruits often exhibits different colors, with black mold being a common occurrence. Aspergillus demonstrates adaptability to changes in the physical, chemical, and biological environment, allowing for rapid growth and development. It possesses a wide range of genes, many of which are associated with the colonization of damaged fruit surfaces Infected or decayed fruits are easily identifiable by the presence of Aspergillus, often manifesting as various colored rot, with black rot being particularly common. *Aspergillus brasiliensis* is a fungus characterized by its conidiophore structure, capable of generating both asexual and sexual spores. It exhibits an aerobic nature, and as a xerophilic species, it can thrive in environments with low moisture levels, including humid conditions. *A. brasiliensis* is predominantly a pathogen affecting plants, there have been rare instances where it can cause illness in humans, specifically in individuals suffering from aspergillosis.

The objective of the present study was to evaluate the potential antifungal activities of *O. sanctum* plant extracted with aqueous and different solvents (Acetone, chloroform and methanol) against *Aspergillus brasiliensis.*

MATERIALS & METHODS

Plant material

Ocimum sanctum was selected as test plant. Fresh leaves of *O. sanctum* were collected from different regions of Udaipur, Rajasthan. The leaves were washed by running water for 2-3 rounds and then rinsed with distilled water. After being washed, the leaves were dried in shaded conditions for approximately 25-30 days. Once dried, they were finely powdered and stored in a sterilized container at room temperature for future use in scientific research.

Extraction of Plant material

The plant material was subjected to extraction using a Soxhlet apparatus. The powdered material was loaded into the Soxhlet extractor, and various solvents (acetone, chloroform, methanol), including aqueous solutions, were added. The solvents

underwent repeated cycles of heating and condensation to extract the active compounds from the powdered material. The resulting solution was collected, and subsequent concentration was performed. An array of concentrations was prepared for the extracted sample, consisting of 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml.

Microorganism

The fungal pathogen employed in the experiment is *Aspergillus brasiliensis.*

Antifungal activity test

The fungal inoculum was evenly spread on Potato Dextrose Agar plates using the surface spread plate technique. These plates were then used for inoculating the microorganism mentioned earlier. The antimicrobial properties of various plant extracts were assessed using the agar well diffusion method, as conducted by Alade and Irobi. After autoclaving, the medium was cooled and transferred to petri dishes. Prior to use, the plates were pre-incubated at 35 degrees Celsius to ensure sterility. A sterile spreader was employed to evenly distribute the test inoculum on the solidified agar surface. Five wells of equal size were created aseptically on the agar plate. Inhibition zone diameters in millimeters (mm) were measured to determine the overall antifungal activity.

RESULT

Determination of Antifungal activity of *Ocimum sanctum*

The aim of the current investigation was to assess the antifungal activity of *Ocimum sanctum* using aqueous and different solvent extracts, namely acetone, chloroform, and methanol. Based on the findings, it was observed that the chloroform extract of *Ocimum sanctum* exhibited the highest zone of inhibition (ZOI) at a concentration of 1.0 mg/ml as shown in Table 3. Additionally, both methanol and acetone extracts displayed antifungal activity at concentrations of 0.4 mg/ml and 0.6 mg/ml, respectively, with ZOI of 10 mm and 12 mm. The investigation into the antifungal activity of the aqueous leaf extract of *Ocimum sanctum* against *Aspergillus brasiliensis* revealed a lack of activity at any concentration tested.

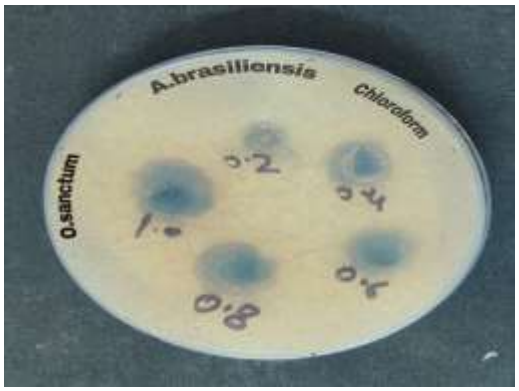


Figure 1. ZOI (Chloroform extract)

Figure 2. ZOI (Aqueous extract)

ZOI for A.brasiliensis (Aqueous extract)

Based on the findings, the aqueous extracts of *O.sanctum* showed no observable zone of inhibition (ZOI) against the tested microorganism, *A. brasiliensis*. (As shown in Table 1.)

Table 1: Tulsi Aqueous leaf extracts at various concentration (0.1mg/ml -1.0mg/ml) and their particular ZOI for *A. brasiliensis*

Concentration Tulsi (mg/ml)	ZOI (mm)
0.2	-
0.4	-
0.6	-
0.8	-
1.0	-

*****ZOI – zone of inhibition

ZOI for A.brasiliensis (Acetone, Chloroform and Methanol)

Table 2: Tulsi Acetone leaf extracts at various concentration (0.1mg/ml - 1.0mg/ml) and their ZOI for *A. brasiliensis*

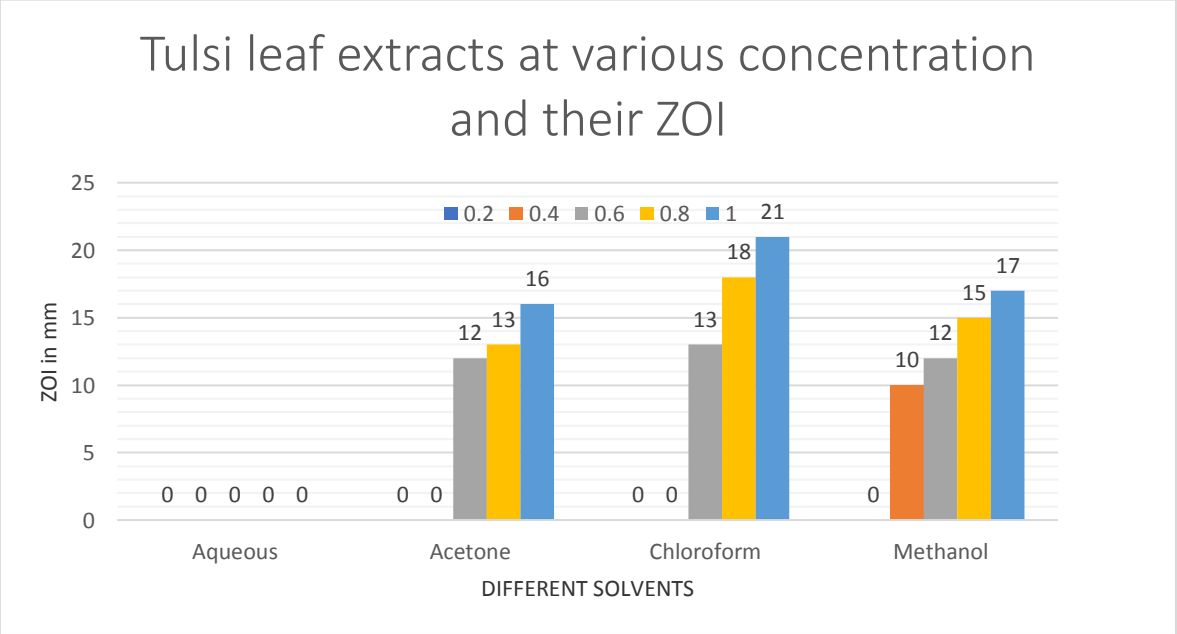
Concentration Tulsi (mg/ml)	ZOI (mm)
0.2	-
0.4	-
0.6	12
0.8	13
1.0	16

Table 3: Tulsi Chloroform leaf extracts at various concentration (0.1mg/ml - 1.0mg/ml) and their ZOI for *A. brasiliensis*

Concentration Tulsi (mg/ml)	ZOI (mm)
0.2	-
0.4	-
0.6	13
0.8	18
1.0	21

Table 4: Tulsi Methanol leaf extracts at various concentration (0.1mg/ml - 1.0mg/ml) and their ZOI for *A. brasiliensis*

Concentration Tulsi (mg/ml)	ZOI (mm)
0.2	-
0.4	10
0.6	12
0.8	15
1.0	17



CONCLUSION

The evaluation of the antimicrobial activity of medicinal plants with pharmacological properties is crucial to harness their potential as a viable source of effective natural drugs. The objective of this study was to investigate the antifungal potency of Tulsi (*O.sanctum*), a medicinal plant, against the pathogenic fungus *A. brasiliensis* using different solvent extracts. The results indicated that the chloroform extract of Tulsi exhibited the highest inhibitory activity compared to the other extracts. At a concentration of 0.4 mg/ml, the chloroform extract did not demonstrate any activity, while the methanol extract showed a zone of inhibition (ZOI) of 10 mm. However, as the concentration increased, the chloroform extract exhibited a larger inhibitory effect compared to the methanol extract (Table 3 and Table 4). These findings indicate that the antifungal potential of Tulsi extracts varies depending on the concentration and the specific solvent used for extraction. Furthermore, the study concludes that *O.sanctum* exhibits noteworthy antifungal potential against *A. brasiliensis*, suggesting its promising value for further exploration in the development of medicinal drugs.

Declaration by Authors
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REFERENCES

1. Aljuboori, A. H. R., Uemura, Y., Osman, N. B., & Yusup, S. (2014). Production of a bioflocculant from Aspergillus niger using palm oil mill effluent as carbon source. *Bioresource technology*, *171*, 66-70.
2. Balakumaran, M. D., Ramachandran, R., Balashanmugam, P., Mukeshkumar, D. J., & Kalaichelvan, P. T. (2016). Mycosynthesis of silver and gold nanoparticles: optimization, characterization and antimicrobial activity against human pathogens. *Microbiological research*, *182*, 8-20.
3. Davis, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science, 264: 375 - 382.
4. Falerio, M.L., Miguel, M.G., Laderio, F., Venâncio, F., Tavares, R., Brito, J.C., Figueiredo, A.C., Barroso, J.G. and Pedro, L.G. (2003). Antimicrobial activity of essential oils isolated from Portuguese endemic species of Thymus. Lett. Appl. Microbiol., 36: 35 - 40.
5. Kalemba, D. and Kunicka, A. (2003). Antimicrobial and antifungal properties of essential oils. Curr. Med. Chem., 10: 813 - 829.
6. Kutyła-Olesiuk, A., Wawrzyniak, U. E., Ciosek, P., & Wróblewski, W. (2014). Electrochemical monitoring of citric acid production by Aspergillus niger. *Analytica Chimica Acta*, *823*, 25-31.
7. Jirovetz, L., Buchbauer, G., Shafi, M.P. and Kaniampady, M.M. (2003). Chemotaxonomical analysis of the essential oil aroma compounds of four different Ocimum species from southern India. Eur. Food Res. Technol., 217: 120 – 124
8. Juliani, H.R. and Simon, J.E. (2002). Antioxidant activity of basil. In: Trends in new crops and new uses. (Janick J and Whipkey A eds.), ASHS Press, Alexandria, V A, pp. 575 - 579.
9. Khanna, N., & Bhatia, J. (2003). Antinociceptive action of Ocimum sanctum (Tulsi) in mice: possible mechanisms involved. *Journal of Ethnopharmacology*, *88*(2-3), 293-296.
10. Naik, L. S., Shyam, P., Marx, K. P., Baskari, S., & Devi, V. R. (2015). Antimicrobial activity and phytochemical analysis of Ocimum tenuiflorum leaf extract. *Int. J. PharmTech Res*, *8*(1), 88-95.
11. Ofor, M. O., Okorie, V. C., Ibeawuchi, I. I., Ihejirika, G. O., Obilo, O. P., & Dialoke, S. A. (2009). Microbial contaminants in fresh tomato wash water and food safety considerations in South-Eastern Nigeria. *Life Sci. J*, *1*, 80-82.
12. Sacchetti, G., Medici, A., Maietti, S., Radice, M., Muzzoli, M., Manfredini, S., Braccioli, E. and Bruni, R. (2004). Composition and functional properties of the essential oil of Amazonian Basil, Ocimum micranthum Willd., Labiatae in comparison with commercial essential oils. J. Agric. Food Chem., 52: 3486 - 3491.
13. Service, R.F. (1995). Antibiotics that resist resistance. Science, 270: 724 – 727
14. Singh, H. (2006). *Mycoremediation: fungal bioremediation*. John Wiley & Sons.

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