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Antifungal activity of leaves and stem extracts of *Ocimum Sanctum*

Garg Praveen[1], Nigam Reni[2]
[1] Department of Science, VITS College, Satna, Madhya Pradesh, India
[2] Department of Biotechnology, AKS University, Satna, Madhya Pradesh, India

Abstract
Antifungal activity of crude methanolic and ethanolic leaves and stem extracts of Ocimum sanctum was evaluated to calculate zone of inhibition. The ethanolic extract of Ocimum sanctum showed more antifungal activity in relative to methanol extracts against two clinical fungus Aspergillus flavus and candida albicans. It is clear from the current study that the Ocimum sanctum represents high antifungal activity against A. flavus in methanolic stem and ethanolic leaf extracts while methanolic leaf and ethanolic stem extract have high antifungal activity against C. albicans. We conclude in this study that Ocimum sanctum has high antifungal activity against both fungus and more antioxidant activity because of the presence of flavonoid content.

Keywords: antifungal activity, HPLC method, *ocimum sanctum*, *quercetin* content

Introduction
Medicinal plants and their valuable products utilized as a source of medicines, which has recognized by the general public and scientist [1]. The utilization of medicinal plants for drug discovery is utilized by human being for various purpose from the starting of human history [2]. *Ocimum sanctum* is belong to lamiaceae family. It is the best known family for therapeutic effects and used as a resource of alimentary herbs [3]. In Ayurveda, *Ocimum sanctum* (Tulsi) has been used for its therapeutic values and described as antiasthmatic and antikaphic drugs [4]. This herb is found on tropical and semitropical area of India. *Ocimum sanctum* can grow in every part of India. It is a 30-75cm high erect herbs, Leaves are 2.5 – 5cm long and 1.6 – 3.2cm broad, elliptical. The inflorescence is verticillate and flowers are in racemes 15-20cm long in close whorls [5]. *Ocimum sanctum* has a various properties such as antioxidant, antibiotic, antiatherogenic, antiulcer, antimicrobial, anti-inflammatory, analgesic, antipyretic, immunomodulatory, and chemopreventive properties [6]. These all properties of *Ocimum sanctum* is because of the presence of phytochemicals. The stems and leaves of tulsi consist of a various phytoconstituents that may have physiological activity, involving alkaloids, flavonoids, glycosides, saponins, triterpenoids, tannins, carbohydrates, and protein [7].

Ocimum sanctum is the commonly and most occurring reservoirs of all household plants in India. Its leaves, seeds and the complete plant is useful. The commom name of is Tulsi and Holi basil. Tulsi extracts are employed in ayurvedic recourses for headaches, common colds, abdomen disorders, inflammation, Cardio vascular disease, numerous kinds of poisoning and protozoal infection. Traditionally, *Ocimum sanctum* is taken in numerous forms such as tea, dried powder or fresh leaf. For centuries old, the dried leaves of tulsi plant have mixed with storage grains to repel insects [8]. The objective of this study was to calculate the antifungal activity of ethanolic and methanolic leaves and stem extracts of *Ocimum sanctum* and used for further medicinal purpose.

Material and methods
Collection of Plant Material: Plant material of *Ocimum sanctum* was collected from the rural area of Chitrakoot (M.P.), in the month of April 2015. These herbs were authenticated by Dr. Rajesh Garg, Department of Botany, Gov. autonomous college, Satna (M.P.). Stems and Leaves of plants were collected for the current study and used it for further experiments.

Preparation of Plant material
The stem and leaves of *Ocimum sanctum* was collected and washed with distilled water and dried in shade. The dried stem and leaves of plant was cut off into small pieces and grounded to powder form. Powdered samples (100gm of each) were extracted with different solvents such as chloroform, ethanol, and methanol using maceration process [9]. Then, the extract was filtered and allowed to evaporate the solvent in a rotary evaporator at 40 deg C and both plant extracts were resuspended in respective solvents for further study.

Fungal culture
There are four fungal strains and their MTCC No. has given below the table 1. The microbes collected from Microbial Type Culture Collection, National Centre for Cell Science, Pune, Maharastra, India. Each fungal species were firstly revived and then subcultured by the use of potato dextrose broth medium then incubated in 37 deg C for 24hr.

Table 1: Name of Pathogenic Fungus

S. No.	MTCC NO.	Microorganism Strains
1	MTCC – 277	*Aspergillus flavus*
2	MTCC – 227	*Candida albicans*
3	MTCC – 16888	*Aspergillus niger*
4	MTCC – 1663	*Alternaria alternate*

Antifungal sensitivity
The antifungal sensitivity test is applied to all microbes *viz; Aspergillus flavus, Aspergillus niger, Candida albicans, Alternaria alterneta* was used under this study with

methanol and ethanol leaves and stem extracts obtained from *Ocimum sanctum*. In this method, potato dextrose agar plates were seeded with tested microbes with the help of spread plate techniques and kept it for 5 minutes. After sometimes prepared the wells in plates and 100mg/ml of extract sample from stock solutions used separately on 6mm diameter wells then incubated for 24 hours at 37 deg C. After incubation period, petri plates were observed to see the sensitivity of extracts sample against test fungi at a specific concentration in the form of zone of inhibition.

Agar well diffusion method

This method is used to determine the antifungal activity of leaves and stem extracts of *Ocimum sanctum* using the standard procedure [10]. In this method, taken a Potato dextrose agar plates with developed fungal culture (sensitive to plant extracts) and prepared wells. There were 3 concentrations used of each extracted plant sample such as methanol and ethanol leaves and stem extracts of *Ocimum sanctum*, which are 25, 50 and 100 mg/ml. Different concentrations of plant extracts samples (25, 50, 100mg/ml) were added into wells with a sterile syringe and allowed for diffuses for 2 hours at room temperature. A similar process repeats with an antibiotic drug fluconazole as a control sample. The plates kept for incubation at 37 deg C for 24 hours and then examined clear zones of inhibition surrounded the wells with a particular concentration of the drug and plant extracts sample. A clear zone of inhibition on the plates indicates the antifungal activity of plant extracts.

Result and discussion

Antifungal sensitivity

The result of antifungal sensitivity of plant extracts was recorded against only in two microbes, *Aspergillus flavus*, and *Candida albicans* while other microbes have no activity (sensitivity) against plant extract sample. The sensitivity of microbes measured in diameter (mm) for methanolic and ethanolic extracts of the plants in terms of zone of inhibition.

Antifungal activity

Antifungal activity of different solvent extracts of *Ocimum sanctum* were obtained and evaluated against two fungal pathogens *A. flavus* and *C. albicans.* These fungal species also tested against standard drug fluconazole, which clearly shown the *C. albicans* is more effective than *A. flavus* in minimum concentration. The result of standard drug and antifungal activity of different solvent extracts of *Ocimum sanctum* has given below in the table no. 2 & 3 (figure 1, 2& 3).

Table 2: Antifungal activity of standard drug on different Antimicrobial agents

S.N	Name of standard drug	Microbes	Zone of inhibition		
1	Fluconazole	*Aspergillus flavus*	25µg/ml	50 µg/ml	100 µg/ml
			15±0.09	18±0.17	20±0.01
		Candida albicans	10 µg/ml	20 µg/ml	30 µg/ml
			16±0.04	20±0.09	28±0.11

Table 3: Antifungal activity of *Ocimum sanctum* extracts on different microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		Leaves (methanolic extract)		
		25mg/ml	50 mg/ml	100mg/ml
1	*Aspergillus flavus*	10±0.12	12±0.15	14±0.11
2	*Candida albicans*	10±0.14	13±0.15	15±0.08
Stem (methanolic extract)				
1	*Aspergillus flavus*	11±0.11	12±0.05	13±0.13
2	*Candida albicans*	8±0.17	9±0.18	10±0.18
Leaves (ethanolic extract)				
1	*Aspergillus flavus*	8±0.12	14±0.12	19±0.11
2	*Candida albicans*	10±0.19	11±0.05	12±0.08
Stem (ethanolic extract)				
1	*Aspergillus flavus*	9±0.12	10±0.15	14±0.14
2	*Candida albicans*	9±0.14	15±0.15	19±0.08

Zone of inhibition (ZOI) values are reported as mean ± SD of three replicates.

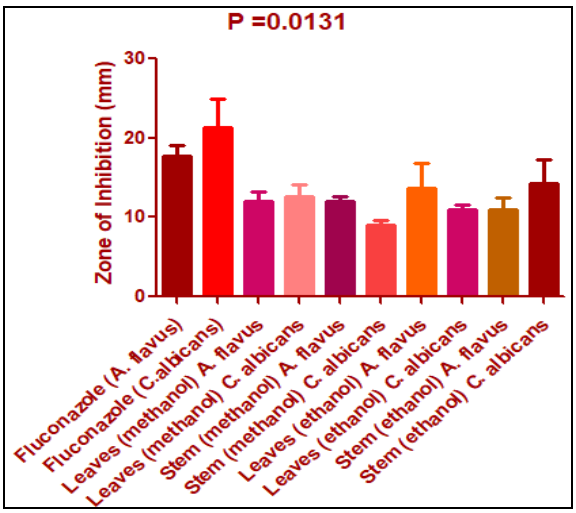

Fig 1: Antifungal activity of different solvent extracts of *Ocimum sanctum* against *Aspergillus flavus* and *Candida albicans*

Table 4

One-way analysis of variance	
P value	0.0131
P value summary	*
Are means Sign if. Different? (P < 0.05)	Yes
Number of groups	10
F	3.270
R squared	0.5954

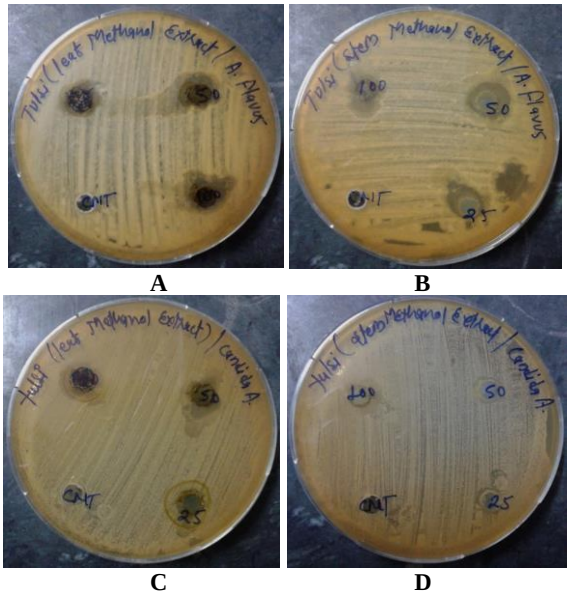

Fig 2: Antifungal activity of (A & B) - Methanol leaf and stem extracts of *Ocimum sanctum* against *Aspergillus flavus* (C & D) - Methanol leaf and stem extracts of *Ocimum sanctum* against *Candida albicans*

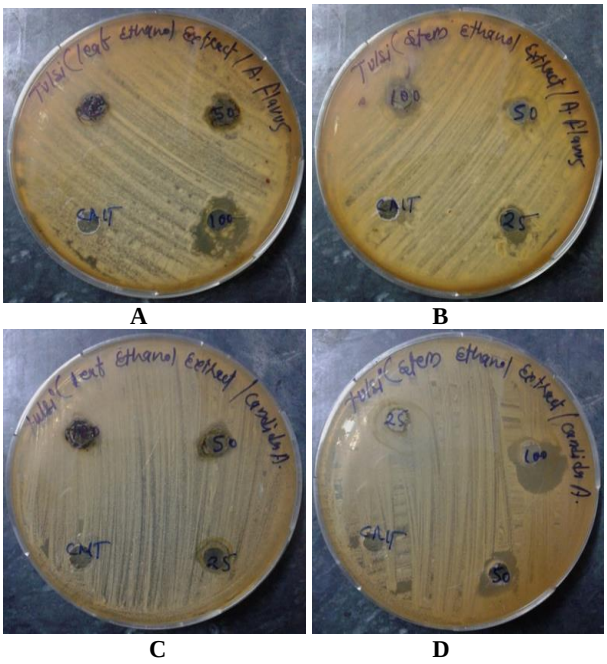

Fig 3: Antifungal activity of (A & B) - Ethanol leaf and stem extracts of *Ocimum sanctum* against *Aspergillus flavus* (C & D) - Ethanol leaf and stem extracts of *Ocimum sanctum* against *Candida albicans*

Medicinal plants contained several phytochemical serves as a precursor to developing less toxic and high effective medicine for controlling the growth of microbes [11, 12]. These phytochemical components have relevant therapeutic applications versus human pathogens including fungi, bacteria or viruses. There are various studies have been done with a variety of plant extracts and screened of antimicrobial activity along with antimicrobial compounds discovered [13, 14]. Therefore, researchers studying the medicinal plants in such a path to find their outcome into pharmaceuticals, neutraceuticals and food Supplements. In the present study, different solvent extracts of both medicinal plants were examined for exploring their antifungal activity towards human pathogenic fungus. The sensitivity of each solvent extracts of the plant was experimented by agar well diffusion method were evaluated. Agar well diffusion method used by many researchers to test the antimicrobial activity [15].

The antifungal activity of methanolic leaves extracts of *Ocimum sanctum* gave a zone of inhibition in diameter ranging from 10±0.12 to 15±0.08mm in size. 100mg/ml concentration of methanolic leaves extracts of *Ocimum sanctum* gave the maximum zone of inhibition is 14±0.11mm and 15±0.08mm in diameter against *A. flavus* and *C. albicans* respectively. 50mg/ml concentration of methanolic leaves extracts of *Ocimum sanctum* gave the intermediate zone of inhibition is 12±0.05mm and 13±0.05mm in diameter against *A. flavus* and *C. albicans* respectively. 25mg/ml concentration of methanolic leaves extracts of *Ocimum sanctum* gave the least zone of inhibition is 10±0.12mm and 10±0.14mm in diameter against *A. flavus* and *C. albicans* respectively.

The antifungal activity of methanolic stem extracts of *Ocimum sanctum* given a zone of inhibition in diameter ranging from 8±0.17 to 13±0.13mm in size. 100mg/ml concentration of methanolic stem extracts of *Ocimum sanctum* gave the maximum zone of inhibition is 13±0.13mm and 10±0.18mm in diameter against *A. flavus* and *C. albicans* respectively. 50mg/ml concentration of methanolic stem extracts of *Ocimum sanctum* gave the intermediate zone of inhibition is 12±0.05mm and 9±0.18mm in diameter against *A. flavus* and *C. albicans* respectively. 25mg/ml concentration of methanolic stem extracts of *Ocimum sanctum* gave the least zone of inhibition is 11±0.11mm and 8±0.17mm in diameter against *A. flavus* and *C. albicans* respectively.

The antifungal activity of ethanolic leaves extracts of *Ocimum sanctum* gave a zone of inhibition in diameter ranging from 8±0.12 to 19±0.11mm in size. 100mg/ml concentration of ethanolic leaves extracts of *Ocimum sanctum* gave the maximum zone of inhibition is 19±0.11 mm and 12±0.08mm in diameter against *A. flavus* and *C. albicans* respectively. 50mg/ml concentration of ethanolic leaves extracts of *Ocimum sanctum* gave the intermediate zone of inhibition is 14±0.12mm and 11±0.05mm in diameter against *A. flavus* and *C. albicans* respectively. 25mg/ml concentration of ethanolic leaves extracts of *Ocimum sanctum* gave the least zone of inhibition is 8±0.12mm and 10±0.19mm in diameter against *A. flavus* and *C. albicans* respectively.

The antifungal activity of ethanolic stem extracts of *Ocimum sanctum* produce a zone of inhibition in diameter ranging from 9±0.12 to 19±0.08mm in size. 100mg/ml concentration of ethanolic stem extracts of *Ocimum sanctum* gave the maximum zone of inhibition is 14±0.14mm and 19±0.08mm in diameter against *A. flavus* and *C. albicans* respectively. 50mg/ml concentration of ethanolic stem extracts of *Ocimum sanctum* gave the intermediate zone of

inhibition is 10±0.15mm and 15±0.15mm in diameter against *A. flavus* and *C. albicans* respectively. 25mg/ml concentration of ethanolic stem extracts of *Ocimum sanctum* gave the least zone of inhibition is 9±0.12mm and 9±0.14mm in diameter against *A. flavus* and *C. albicans* respectively.

Thus, It is clear that *Ocimum sanctum* represents high antifungal activity against *A. flavus* in ethanolic leaf extracts followed by ethanolic stem and methanolic leaves extracts while methanolic leaf and ethanolic stem extract have high antifungal activity against *C. Albicans*.

It is clear from the current study that the *Ocimum sanctum* represents high antifungal activity against *A. flavus* in methanolic stem and ethanolic leaf extracts while methanolic leaf and ethanolic stem extract have high antifungal activity against *C. albicans.*

There are many researchers have been studied on phytochemicals and their medicinal properties. These phytochemical compounds have therapeutic properties against different types of microbes and it is not surprising that these medicinal plants are used in traditional form by old people to cure fungal and bacterial infections. *Ocimum sanctum* has antifungal activity against tested pathogens cause of the presence of phytoconstituents. The present study thus pointed out that *Ocimum sanctum* are effective against fungal infections caused by *A. flavus and C. albicans.*

Conclusion

The current study concluded that different solvent extracts could be seen as a better source of rich phytochemical compounds. Thus, there is essential to use these solvents for qualitative analysis of plants. This study reviews the traditional knowledge, ethnomedicinal, pharmacological and therapeutic applications of the plant *Ocimum sanctum.* The results come out in this study suggested that the phytochemicals constituents may be responsible for the antifungal activity. Efficacy of leaves and stems of methanolic and ethanolic extracts of *Ocimum sanctum* is because of the secondary metabolites such as flavonoids that may be of grand usage for the progression of pharmaceutical industries as a therapeutic material against various diseases. The ethanol, methanol extracts of plants possess relevent inhibitory effect against tested pathogens.

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