Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 11 [5]APRIL 2022 : 219-223

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial and Antifungal Activity of Ethanolic and Methanolic Extract Obtained from *Ocimum sanctum* Linn.

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ABSTRACT
Holy basil Ocimum sanctum L. has taken an important place in traditional values for India. The present study investigates the antibacterial and antifungal activity of ethanolic and methanolic plant extract. Plant parts like leaves, steam and roots were collected from local region of Uttarakhand. The antifungal activity of methanolic leaves, stem and seed extracts of Ocimum sanctum gave a zone of inhibition in diameter ranging from 10 to 15, 11 to 13 and 14 to 21mm, respectively. The obtained data from Ocimum sanctum L. confirmed its wide application for therapeutic purpose in alternative therapy.
***Key words**: Ocimum sanctum L., Antibacterial activity, Antifungal activity, Secondary metabolites.*

Received: 19.02.2022 Revised: 12.03.2022 Accepted: 30.03.2022

INTRODUCTION

Tulsi (*Ocimum sanctum)*, commonly known as holy basil in English and sacred basil in Hindi whereas Vishnu Priya in Sanskrit the literal meaning of its Latin name. It possesses potential therapeutic characters and use to treat many diseases like bronchial asthma, chronic fever, colds cough, malaria, dysentery, convulsions, diarrhea, arthritis, emetic syndrome, skin diseases, insect bites etc., and also used in the treatment of gastric, hepatic, cardiovascular and immunological disorders etc. It also heals various clinical conditions like eye disorders (glaucoma, cataract, & chronic conjunctivitis), catalepsy, snake and scorpion bites etc [13].

Tulsi shows different activity against bacterial, viral and fungal components as the aqueous fraction and methanolic fraction of *O. sanctum* shows antifungal activity against several dermatophytic fungus [6]. Linolenic acid and fixed oil, present in Tulsi act as anti-inflammatory as they have the ability to block cyclooxygenase and lipoxygenase pathways of arachidonic acid also increase in metabolism has been seen [12]. Leaves of Tulsi help to prevent and reduce physical and mental stress because of having powerful adaptogenic property [3]. Tulsi have some antibacterial properties as it is a powerful herb used by humans from old times having properties of aqueous alcoholic, chloroform extract and oil obtained from leaves [8]. Benzene extract of *O. sanctum* leaves treated with albino rats decreased the total sperm count and sperm motility results androgen deprivation due to anti androgenic property of *O. sanctum.* Sperm testosterone level increased whereas level of FSH and LH sperm count were reduced in rabbits [1].

Many antibiotics are developed for the treatment of bacterial infections. Scientists search for antimicrobial substances from various sources such as medicinal plants, one of them is "Tulsi" having diverse uses and one of the holiest and commonly used medicinal plant many of them used in fungal infections and fresh juice used treatment of several diseases like bronchitis and skin diseases [10].

Outbreaks of diarrhea are common in communities living in precarious conditions with poor sewerage and hygiene. A number of food types have been linked to outbreaks and act as the carriers of infectious microbes. Under such conditions, diarrhea is a common occurrence after eating contaminated fish products. Illnesses arise because the microbial causal agents, often native to the fish itself, have not been adequately controlled, or arise as a consequence of incorrect handling and or storage during industrial processing [4]. Illnesses causing gastroenteritis and diarrhea are primarily associated with enteric bacteria. Enteric bacteria are responsible for high mortality rates in numerous developing countries with as many as 50,000 people dying daily as consequence of infection [5]. Plant remedies are increasingly being recognized by scientists as a very important low cost alternative to industrially-produced antibiotics which are not available to all who need them because their high price [3].

Hence there is need for discovery of new safer and effective drug. Medicinal herbs could be alternate source for treatment of these pathogens. Some essential oil of *O. sanctum* demonstrated highly active antifungal agents against dermatophyte. The major active substances in this oil are Eugenol and its derivatives [2]. Investigation was undertaken to screen whole part extract of aromatic *O. sanctum* for their antibacterial activity against bacteria and whole part of Tulsi.

MATERIAL AND METHODS

Reagents and chemicals

Chloroform, ethanol and methanol solvents and chemicals used were purchased from Merck and Sigma–Aldrich, anhydrous sodium carbonate, Aluminium trichloride (AlCl3), sodium nitrite, sodium chloride potassium acetate, ferric chloride, butylated hydroxytoluene (BHT), diethyl ether, ammonia solution, acetone, ethanol, hydrochloric acid, sodium hydroxide, phosphate buffer, potassium ferricyanide, ammonium molybdate, sodium phosphate, trichloroacetic acid, glacial acetic acid and sodium nitroprusside. All the chemicals used in this study were of analytical grade.

Procurement of plant material

The whole plant of *O. sanctum* (Tulsi) was used for the experimental purpose in present work. The collected plant was identified according to various literatures [7].

Preparation of plant material

Leaves, seeds and roots will be washed with distilled water to remove any dirt particles. The washed parts will be shade - dried for 10-12 days and will be converted into coarse powder by grinder and then stored at room temperature for further processing [7].

Extraction of metabolite from *O. sanctum*

The extraction will be done using Soxhlet method. In this method powdered plant material will be subjected to successive extraction with Chloroform, Ethanol, Methanol [6]. The obtained extracts will be filtered out, distilled off on water bath and stored under refrigeration for future processing.

Collection and preparation of test organisms

Test organisms such as *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA) and *Escherichia coli* will be received from university department and reconfirmed by Gram staining and sub culturing in appropriate selective media [7].

Preparation of Mcfarl and standard

The inoculums of test bacteria will be prepared by making a direct broth suspension of isolated colonies and turbidity will be adjusted to achieve a value equivalent to a 0.5 McFarland turbidity standard [9].

In-vitro antibacterial activity of *O. sanctum* extracts

Antibacterial activity will be performed using agar well diffusion method. The test bacteria will be spread on MHA plates. Well will be cut from agar plates with a sterile cork borer. Whole plant extract with suitable positive and negative controls will be applied in wells followed by incubation at 37^{o}C for 24 hrs and observe the zone of inhibition [11].

RESULTS AND DISCUSSION

Chromatographic examination of *O. sanctum* extract

TLC method performed with standard flavonoid components such as quercetin and plant extract samples, in which total flavonoid content (TFC) presented. The chromatographic result of standard quercetin and chromatogram of quercetin and plant sample extract has mentioned in table 1. TLC plates were observed under Normal light, Short UV and Long UV. The similar Rf value (0.78) of quercetin and plant extract sample was recognized.

Table 1: Thin Layer Chromatography of *Ocimum sanctum* extract

S.No.	Compound	Extract	Rf Value
1	Quercetin	Toluene: Ethyl acetate: Formic acid	0.78

A chromatogram was observed under normal light, short U.V. light and long U.V. light. A developed chromatogram consisted of four lanes:

Lane No. 1 - Standard Quercetin

Lane No. 2 - Methanolic leaves extracts of Ocimum sanctum

Lane No. 3 - Ethanolic leaves extracts of Ocimum sanctum

According to Rf value, developed chromatogram of all extract sample confirmed that quercetin present in methanolic leaves extract of *Ocimum sanctum*. While ethanolic leaves extract of *Ocimum sanctum* has not shown the presence of quercetin. Chromatogram analysis of quercetin and extracts sample encompasses of Toluene, Acetic acid, Formic acid (5:4:1) as mobile phase.

Antifungal Activity of Phytochemical Extracts of *O. sanctum*

Antifungal activity of extract was performed and for this study, the culture of fungal species was isolated from department culture collection centre. These fungal species were cultured in potato dextrose broth medium and kept in an incubator at 37 ^{o}C for 5-7 days.

Under the present study, fungal disc were used to prepare lawn and metanolic and ethanolic extracts of *O. sanctum* were studied at 25, 50 and 75, 100mg/ml concentration to check the sensitivity of microbes against phytochemical extracts by using methods of agar well diffusion. Table 2 showed that the antifungal sensitivity of both plant extracts was recorded against only in two microbes, *Aspergillus flavus* and *Candida albicans*. The sensitivity of microbes measured in diameter (mm) for methanolic and ethanolic extracts of the plants in terms of zone of inhibition.

Table 2: Zone of inhibition (in mm) against fungal test organisms in *O. sanctum* extracts

Extract/ Standard		Concentration (mg/ml)	*Aspergillus flavus*	*Candida albican*
Methanol	Leaves	25	10	10
		50	12	13
		100	14	15
	Stem	25	11	8
		50	12	9
		100	13	10
	Seed	25	14	11
		50	19	15
		100	21	18
Ethanol	Leaves	25	8	10
		50	14	11
		100	19	12
	Stem	25	9	9
		50	10	15
		100	14	19
	Seed	25	13	15
		50	17	18
		100	19	20
KET		25	16	15
		50	17	18
		100	22	21
Abbreviations: (mg/ml)= milligram/milliliter; (KET)= Ketoconazole				

Antimicrobial activity from natural sources has been investigated, after many effort and attention it has been identified the phytochemical compound that acts as an antimicrobial agent to replaced synthetic ones. Medicinal plants contained several phytochemical serves as a precursor to developing less toxic and high effective medicine for controlling the growth of microbes [1, 8]. Therefore, researchers studying the medicinal plants in such a path to find their outcome into pharmaceuticals, neutraceuticals and food Supplements.

Earlier results showed that higher plants consist of phenolic compounds, alkaloids, and flavonoids as secondary metabolites are relevant for antimicrobial activity [9]. The variety of medicinal plants like Giloy, Tulsi and others are being used for the treatment of inflammation, wound healing, toothache, cough, antiseptics expectorant, stomatitis and some fungal infection like candidiasis since olden time.

The result of antifungal activity has mentioned in the table 2, whereas three concentration of the solvent extract of plant sample was used, from which higher concentration (100mg/ml) of plant extract sample exhibited maximum zone of inhibition against fungal species. The antifungal activity of methanolic leaves, stem and seed extracts of *Ocimum sanctum* gave a zone of inhibition in diameter ranging from 10 to 15, 11 to 13 and 14 to 21mm, respectively. 100mg/ml concentration of methanolic seed extracts of *Ocimum sanctum* gave the maximum zone of inhibition is 21mm and 19mm in diameter against *A. flavus* and *C. albicans,* respectively.

The highest zone of inhibition of plant extracts sample and standard solution (ketoconazole) represents the high antifungal activity against both clinical fungi such as *A. Flavus* and *C. albicans*. *A. flavus* has shown

maximal zone of inhibition in minimum concentration than *C. albicans* against standard drug (fluconazole). It is clear that *Ocimum sanctum* represents high antifungal activity against *A. flavus* in metanolic seed extract followed by metanolic leaf extracts followed by methanolic stem.

The reason for higher antifungal activity in both extracts due to the part of high concentration of antifungal compounds such as flavonoids, alkaloids and phenols. All the plants extracted with solvents showed antifungal activity. However, the plants from different locations differ in their activities against the clinical fungus. The concentration of secondary metabolite which leads to variations in antimicrobial activity as has been seen in the present investigation.

Antibacterial Activity of Phytochemical Extracts of *O. sanctum*

In vitro antimicrobial activity of *O. sanctum* was evaluated using agar well diffusion method. Antibacterial activity was performed against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Standard drugs tetracycline was used as a standard antifungal agent while solvents which were used for extraction were used as negative control.

Results of antibacterial activity of different extracts of *O. sanctum* showed that as the concentration of extracts increased from 2 mg/ml to 8 mg/ml the antibacterial activity also increased. Against *Escherichia coli*, maximum zone of inhibition was observed with methanol seed extract (17 mm) followed by methanol leave extract (16 mm). Similarly, against *Staphylococcus aureus*, maximum zone of inhibition (16 mm) was observed with methanolic and ethanolic seed extract (Table 3).

Table 3: Zone of inhibition (in mm) against bacterial test organisms in *O. sanctum* extracts

Extract/ Standard		Concentration (mg/ml)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
Methanol	Leaves	2	9	10	10
		4	10	11	12
		6	12	13	13
		8	13	14	15
	Stem	2	10	9	10
		4	12	10	11
		6	13	11	12
		8	14	12	13
	Seed	2	10	13	13
		4	11	14	14
		6	14	15	15
		8	15	16	17
Ethanol	Leaves	2	8	9	10
		4	9	11	11
		6	11	12	13
		8	12	13	14
	Stem	2	10	10	10
		4	11	10	11
		6	13	12	13
		8	14	14	15
	Seed	2	8	10	9
		4	11	12	12
		6	13	14	15
		8	15	16	16
TET		50	18	24	16
Abbreviations: (mg/ml)= milligram/milliliter; (TET)= Tetracycline					

Minimum inhibitory concentration

The MIC values of metanlic and ethanolic extracts are shown in Table 4. Results showed that against *A. flavus*, *O. sanctum* seeds methanol seed extract had lowest MIC value (5 mg/ml) whereas against *C. albicans* lowest and similar MIC value was observed with methanol seed extract (5 mg/ml). *E. coli* shows the minimal MIC of 1.25 mg/ml in methanolic and ethanolic seed extract.

Table 4: MIC (mg/ml) of *O. sanctum* extracts against test organism

Extract/ Standard		*B. subtilis*	*S. aureus*	*E. coli*	*A. flavus*	*C. albican*
Methanol	Leaves	2.5	5	1.25	7.5	10
	Stem	5	7.5	2.5	5	7.5
	Seed	2.5	5	1.25	5	7.5
Ethanol	Leaves	2.5	7.5	1.25	7.5	12.5
	Stem	5	7.5	2.5	7.5	7.5
	Seed	2.5	2.5	1.25	5	5

CONCLUSION

Because of emergence of multiple drug resistance in human pathogenic organisms, search for new antimicrobial substances from alternative sources including plants is gaining momentum. There are various reports available that indicate tulsi is a potential source for different biological activity. Hence present work can be seen as a potential source of useful drugs. Further studies are in progress of this plant for the purpose of isolation, identification, characterization and explanation of the structure of the phytochemical compounds.

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CITATION OF THIS ARTICLE

U Singh, PK Upadhyay and A K Singh. Antibacterial and Antifungal Activity of Ethanolic and Methanolic Extract Obtained from *Ocimum sanctum* Linn., Bull. Env. Pharmacol. Life Sci., Vol 11`[5] April 2022: 219-223