



Research Article

ASSESSMENT OF BACTERIAL GROWTH INHIBITION PROPERTY AND PHYTOCHEMICAL ANALYSIS OF *OCIMUM SANCTUM* L. LEAF EXTRACT

Malay Kr Das¹, Manisha Mandal², Shyamapada Mandal^{1*}

¹Laboratory of Microbiology and Experimental Medicine, Department of Zoology, University of Gour Banga, Malda, India

²Department of Physiology, MGM Medical College and LSK Hospital, Kishanganj, Bihar, India

*Corresponding Author Email: samtropmed@gmail.com

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ABSTRACT

Tulsi plant, *Ocimum sanctum* L., is famous for its therapeutic potentiality. The present study investigates the antibacterial activity of *O. sanctum* leaf extracts, and the bioactive components present in the extracts. The antibacterial activity of ethanolic leaf extract of *O. sanctum*: dark variety; Krishna tulsi (DOSE) and bright variety; Rama tulsi (BOSE), and aqueous leaf extract of dark variety (AqDOS) and bright variety (AqBOS) of *O. sanctum* was determined by *in vitro* methods against gram-negative and gram-positive clinical bacterial isolates. Phytochemical screening of the extracts was done qualitatively, and thin layer chromatography (TLC) was performed using n-hexane-ethyl acetate mobile phase. The ethanolic extracts showed more antibacterial activity as compared to aqueous extracts in terms of ZDI (zone diameter of inhibition); the ZDI of DOSE ranged 20 – 28 mm for gram-positive, and 14 – 23 mm for gram-negative bacteria, whereas in case of BOSE the ZDIs ranged 11 – 22 mm and 12 – 18 mm, respectively, for gram-positive and gram-negative bacteria. The R_f values of phytochemicals detected on the TLC plate, ranged 0.06 - 0.94. The *O. sanctum* leaf extracts had broad spectrum antibacterial activity and found rich source of phytochemicals, which thus, be utilized against a broad range of bacterial infection.

Keywords: *Ocimum sanctum*, antibacterial activity, zone diameter of inhibition, phytochemicals, thin layer chromatography, R_f values.

INTRODUCTION

The medicinal plants possess therapeutic potentiality because of the presence of several chemical components of varied composition that are found, as the secondary metabolites, in plant parts like leaves, fruits, stem, root, flower and seeds¹. India is a rich source of such kind of medicinal plants. A majority of the world's population, especially in the developing countries, depends on herbal medicines to meet the primary health needs².

Tulsi, which is called the holy basil, and scientifically known as *Ocimum sanctum* L. (= *Ocimum tenuiflorum*), belongs to the family Lamiaceae³, and is popular one for its therapeutic potentiality to treat many diseases⁴. The plant is native to the Indian Subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics⁵. Two varieties of *O. Sanctum*, commonly found in the Indian include dark variety or the Krishna tulsi and bright variety or the Rama tulsi^{2,6,7}.

In the traditional system of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *O. sanctum* have been recommended for the treatment of several health disorders including diarrhoea and dysentery⁸. It has been demonstrated that the stem and leaves of *O. sanctum* possess several bioactive compounds such as saponins, flavonoids, triterpenoids, tannins⁹ as well as phenolic compounds¹⁰, which have great therapeutic importance in curing many diseases, and are also responsible for antimicrobial activity^{11,12}. Possessing enormous number of bioactive components^{6,13}, *O. sanctum* potentiality inhibit the growth of several bacterial pathogens including *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*

(*P. aeruginosa*), *Salmonella enterica* serovar Typhi (*S. typhi*), *Staphylococcus aureus* (*Staph. aureus*) and *Bacillus subtilis*^{4,14-16}.

In our region, there is abundance of both the varieties of *O. sanctum* (dark and bright), but no scientific report has been documented on the antibacterial activity of this plant available in the local niches. Therefore, the present study was designed to explore the antibacterial potentiality of *O. sanctum* leaf extracts and to screen the bioactive constituents contained in them.

MATERIAL AND METHODS

Bacterial strains

The randomly selected bacteria, for the current study, included gram-negative (*E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *P. aeruginosa*, *S. typhi*, *Acinetobacter baumannii*) and gram-positive (*Staph. aureus* and *Bacillus cereus*) clinical isolates. The control strains used were *E. coli* MTCC 443 and *Listeria monocytogenes* MTCC 657.

Collection of plant materials

The leaves from two indigenous variety of *O. sanctum*: Krishna tulsi (dark variety) and Rama tulsi (bright variety) were collected from Khanta village of Uttar Dinajpur district, West Bengal state (India). The leaves were washed properly with distilled water, shed dried and grinded using an electronic grinding machine, and stored in airtight containers at room temperature for extract preparation as per the requirement.

Preparation of extract

For the preparation of *O. sanctum* leaf extracts, ethanol and water were used as the solvents. The ethanolic extracts were prepared following the protocol described earlier¹⁷. Twenty five gram of powdered leaves was dissolved in 200 ml of ethanol for 48 h with shaking at regular intervals. The liquid phase of the extract was filtered with Whatman No.1 filter paper, following filtration through a sterile cheese cloth, and stored in refrigerator at 4°C for further use. The aqueous extracts were prepared by dissolving the 25 g of the powder, of each variety, in 200 ml distilled water, and boiled for 30 min, in water bath, filtered as mentioned above after cooling. The concentration of all the extracts prepared: ethanolic leaf extract of *O. sanctum* dark variety (DOSE) and bright variety (BOSE), and aqueous leaf extract of *O. sanctum* dark variety (AqDOS) and bright variety (AqBOS), in the stock solution, was 0.125 mg/μL.

Antibacterial activity of plant extract

The antibacterial activity of the plant extracts was performed by disk diffusion method¹⁸, using nutrient agar medium; the detail of the method has been described elsewhere¹⁷. The extract concentrations used were 3.75 and 6.25 mg/disc. The zone diameter of inhibition (ZDI) values were recorded and interpreted according to Mandal *et al.*¹⁹: the ZDIs ≥ 7 mm were indicative of sensitivity of the test bacterial isolates to the *O. sanctum* leaf extracts.

Phytochemical screening

To ascertain the presence of specific bioactive compounds in *O. sanctum* ethanolic and aqueous leaf extracts, different methods were followed, as described by Radhakrishnan *et al.*,²⁰ for quinones, phenols, steroids, terpenoids and flavonoids, by Ayoola *et al.*,²¹ for cardiac glycosides, and by Joshi *et al.*²² and Devi *et al.*⁴ for anthraquinone glycosides and saponins, respectively.

Thin layer chromatography

The *O. sanctum* leaf extracts (DOSE, BOSE, AqDOS and AqBOS) were applied in spot forms, using glass capillaries, on a precoated silica gel 60F₂₅₄ TLC plates (Merck, India), with a developing distance of 1.5 cm, among four tracks. The plate was developed in hexane-ethyl acetate (8:2 v/v) solvent system in a developing chamber. The TLC plate was air dried, after removing it from the developing chamber, when the solvent moved a predetermined distance of 15 cm from the origin. The colour components detected on the TLC plate were photographed in visible light. The TLC plate was then placed in an iodine chamber and examined under visible as well as UV light (365 nm). The TLC fingerprints of *O. sanctum* leaf extracts were recorded by photography. The movement of the compounds was expressed with their retention factor (R_f) values, calculated according to Gujjeti and Mamidala²³:

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

RESULTS AND DISCUSSION

The *O. sanctum*, in India, remains a famous and the most sacred plant that played an important role in ancient medicine to modern research due to its remedial properties against the wide array of illnesses^{8,24}. In the current study, following disc diffusion technique, the antibacterial activity of all the test extracts has been determined (Figure 1). The ethanolic *O. sanctum* leaf extracts (DOSE and BOSE) had superior growth inhibition activity compared to the aqueous extracts, AqDOS and AqBOS (Figure 2); the ZDIs of DOSE ranged 20 – 28 mm for gram-positive and 14 – 23 mm for gram-negative bacteria, while the ZDIs of BOSE ranged 11 – 22 mm and 12 – 18 mm, respectively, for gram-positive and gram-negative bacteria. The research on different parts of various plants has been increased, in recent times, all over the world, as the consequence of emerging multidrug resistance among bacterial pathogens¹⁹. The *O. sanctum* leaves have displayed equal efficacy against the gram-negative as well as gram-positive bacteria¹⁴. The methanolic extract of *O. sanctum* showed strong antibacterial activity against *Staph. aureus* and *Staph. saprophyticus* (ZDI: 20 mm) as compared to other test bacterial isolates²⁵. Krishnan and Nair²⁶ reported that the ethanolic leaf extract of *O. sanctum* had highest ZDI (19 mm) at 0.05 g concentration against *P. aeruginosa*. Worth mentioning, the *O. sanctum* leaf extracts displayed concentration dependent antibacterial activity against both gram-positive and gram-negative test bacteria. The antimicrobial activity of ethanolic *O. sanctum* leaf extract has been reported to be augmented with increased concentration of the extracts as well as the time of exposure, and found more efficacious than the aqueous *O. sanctum* leaf extracts²⁷. The variation in antimicrobial activity of a plant was due to the presence of varied quantity and number of secondary metabolites in different solvent extracts^{28,29}.

The medicinal plants are rich in secondary metabolites of therapeutic importance³⁰. Herein, the ethanolic (DOSE and BOSE) and aqueous (AqDOS and AqBOS) extracts of *O. sanctum* were screened for the presence of bioactive phytochemicals. All the *O. sanctum* extracts were tested positive for cardiac glycosides, anthraquinone glycosides, steroids and quinones, terpenoids were not found in BOSE, while saponins and phenols were detected, respectively in the ethanolic (DOSE and BOSE) and aqueous (AqDOS and AqBOS) extracts (Table 1). Prasad *et al.*³¹ reported the presence of cardiac glycosides, anthraquinones, terpenoids, flavonoids, tannins, saponins and lignins in *O. sanctum*. Various extracts of *O. sanctum* leaves showed the presence of alkaloids, glycosides, volatile oils, flavanoids, terpenoids and tannins³². Reddy *et al.*³³ demonstrated the presence of saponins, alkaloids, flavonoids, cardiac glycosides, carbohydrates, terpenoids and tannins in ethanolic extract of *O. sanctum* leaves. The *O. sanctum* aqueous and methanolic leaf extracts, which were screened to contain steroids, alkaloids, tannins and reducing sugars, had growth inhibitory activity against *Staph. aureus*, *E. coli* and *Pr. mirabilis*³⁴. Haniffa and Shanthi³⁵ documented the presence of tannins and flavonoids in the *O. sanctum* methanolic leaf extract showing growth inhibitory activity against *Aeromonas hydrophila* (ZDI: 15 mm). In the current study the presence of various bioactive compounds in *O. sanctum* leaf extracts might be responsible in displaying broad spectrum antibacterial activity (against both gram-negative and gram-positive pathogenic bacteria).

Table 1: Phytoconstituents present in the test plant extracts

Phytoconstituents	Plant extracts			
	DOSE	BOSE	AqDOS	AqBOS
Flavonoids	-	-	-	-
Cardiac Glycosides	+	+	+	+
Anthraquinone Glycosides	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	-	+	+
Quinones	+	+	+	+
Phenols	+	+	+	+
Sapponins	+	+	-	-

DOSE: ethanolic leaf extract of *O. sanctum*: dark variety; BOSE: ethanolic leaf extract of *O. sanctum*: bright variety; AqDOS: aqueous leaf extract of *O. sanctum*: dark variety; AqBOS: aqueous leaf extract of *O. sanctum*: bright variety; +: presence of the compound; -: absence of the compound.

Table2: R_f values of various phytocomponents in *O. sanctum* leaf extracts detected on TLC plate

Plant Extract	Fraction Number	Visibility of spot		R _f value
		UV Light	Visible Light	
DOSE	1	Visible	Visible	0.12
	2	Visible	Visible	0.22
	3	Visible	Visible	0.30
	4	Visible	Visible	0.43
	5	Visible	Visible	0.52
	6	Visible	Not Visible	0.58
	7	Visible	Visible	0.69
	8	Visible	Visible	0.80
	9	Visible	Not Visible	0.86
	10	Visible	Visible	0.94
AqDOS	1	Visible	Not Visible	0.29
	2	Visible	Not Visible	0.39
	3	Visible	Not Visible	0.84
BOSE	1	Visible	Not Visible	0.06
	2	Visible	Visible	0.12
	3	Visible	Visible	0.24
	4	Visible	Visible	0.30
	5	Visible	Visible	0.38
	6	Visible	Visible	0.41
	7	Visible	Visible	0.49
	8	Visible	Not Visible	0.56
	9	Visible	Visible	0.64
	10	Visible	Visible	0.76
	11	Visible	Not Visible	0.86
	12	Visible	Visible	0.93
AqBOS	1	Visible	Not Visible	0.29
	2	Visible	Not Visible	0.39
	3	Visible	Not Visible	0.84

DOSE: ethanolic leaf extract of *O. sanctum*: dark variety; AqDOS: aqueous leaf extract of *O. sanctum*: dark variety; BOSE: ethanolic leaf extract of *O. sanctum*: bright variety; AqBOS: aqueous leaf extract of *O. sanctum*: bright variety; R_f: retention factor

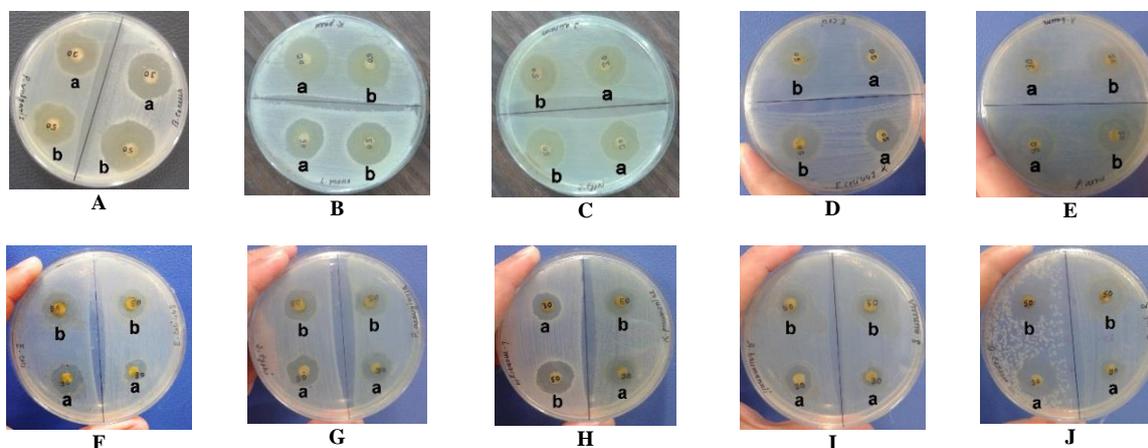


Figure 1: Antibacterial activity of *O. sanctum* leaf extracts; A-E; dark variety ethanolic extract; F-J; bright variety ethanolic extract. a= 3.75 mg; b= 6.25 mg. The clear halos around each disc on the plates are indicative of growth inhibitory action of the extracts.

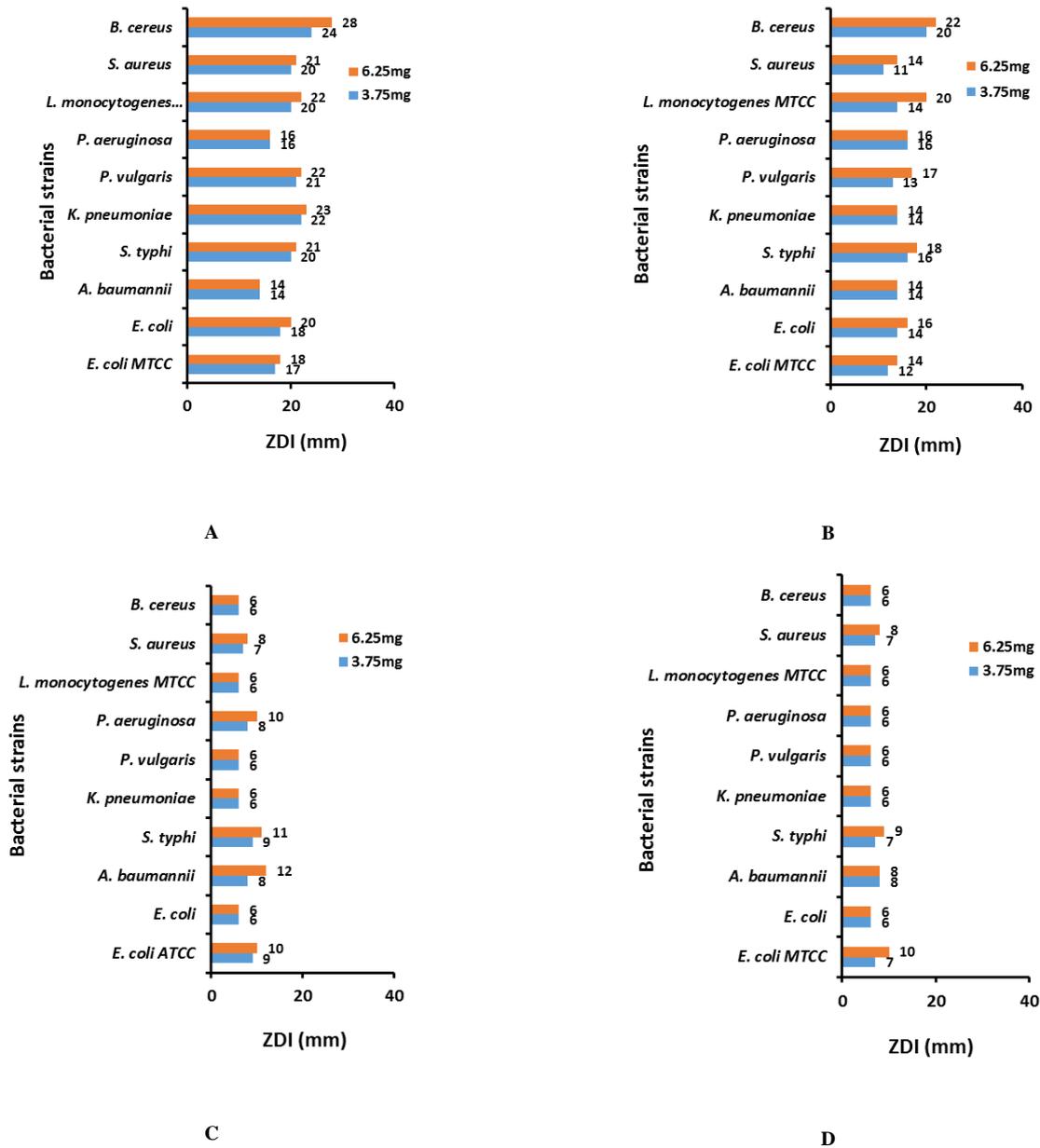


Figure 2: The ZDI (Zone diameter of inhibition) values due to the action of *O. sanctum* leaf extracts by disc diffusion method; A= ethanolic extract of dark variety; B= ethanolic extract of bright variety; C= aqueous extract of dark variety; D= aqueous extract of bright variety.

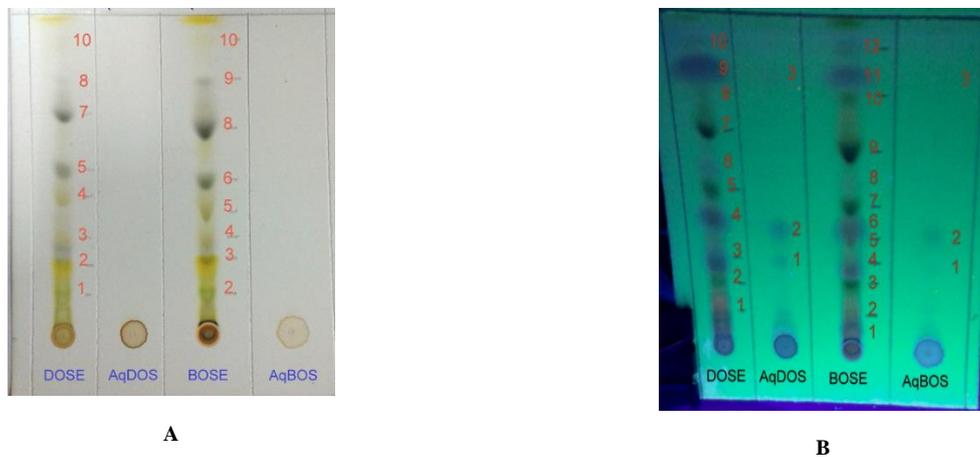


Figure 3: TLC chromatogram of *Ocimum sanctum* leaf extracts in n-hexane-ethyl acetate solvent system. A: spots detected in visible light; B: spots detected in UV light; DOSE: ethanolic leaf extract of *O. sanctum*: dark variety; AqDOS: aqueous leaf extract of *O. sanctum*: dark variety; BOSE: ethanolic leaf extract of *O. sanctum*: bright variety; AqBOS: aqueous leaf extract of *O. sanctum*: bright variety.

Thin layer chromatography (TLC) is important for the separation and detection of phytoconstituents present in plant extracts. The most of the spots, on the TLC plate, from DOSE and BOSE, in the current study, were detected in visible as well as UV light, while the spots from AqDOS and AqBOS were seen in UV light only (Figure 3). The *O. sanctum* ethanolic extracts showed more number of spots, with R_f values 0.06-0.94, than the *O. sanctum* aqueous extracts, conferring R_f values 0.29 - 0.84 (Table 2). The TLC chromatogram revealed the presence of two components (R_f values: 0.13 and 0.63) in *O. sanctum* leaf ethanolic extract, and such components were found active against bacterial pathogens³⁶. Reddy *et al.*³³, following TLC analysis in 'chloroform-methanol-water' (10:10:3) solvent system, demonstrated flavonoids content (R_f value: 0.82) in *O. sanctum* leaf ethanolic extract. The chromatographic variation, in TLC study, might be due to the difference in polarity of solvent system^{37, 38}, variation in the methods of extraction and the solvents used in the extraction process, stage of maturity of the leaves, seasonality as well as the geographical variation.

CONCLUSION

The *O. sanctum* (black and bright variety) contained several bioactive phytoconstituents: phenolics, tannins, terpenoids, saponins, quinones and glycosides, in the leaf ethanolic as well as aqueous extracts, of which the former had excellent antibacterial activity against both gram-negative and gram-positive human pathogenic bacteria. The presence of such bioactive compounds in the leaf extracts of the test plant were validated by TLC chromatogram, and thus, the capacity of antibacterial activity of the plant might be attributed to such active compounds, alone or in combination. Therefore, the *O. sanctum* leaves, which are enormously available in the local niches from this part of the globe, might be utilized as the source of alternative and non-antibiotic agents in order to combat bacterial infection.

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