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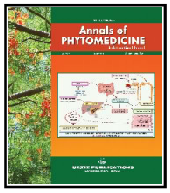

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Antibacterial activity of tulsi (*Ocimum* spp.) against plant pathogenic bacteria

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Abstract

Antimicrobial activities of two tulsi species, *Ocimum sanctum* L. and *Ocimum canum* Sim. extracts were evaluated against two bacterial plant pathogens, *Ralstonia solanacearum* and *Xanthomonas oryzae*. Agar well diffusion method was followed for antimicrobial activity assay. Extracts of methanol, n-hexane, chloroform and ethyl acetate were used with five different concentrations, *viz.*, 10, 20, 30, 40 and 50 mg/ml of 100% DMSO for both *Ocimum* species. Out of the different solvent extracts of *O. sanctum*, the chloroform extract showed the highest zone of inhibition (*i.e.*, 9.66 mm) at 50 mg/ml against *X. oryzae* and the same concentration of methanolic extract showed the highest zone of inhibition (*i.e.*, 7.16 mm) against *R. solanacearum*. Among the different solvent extracts of *O. canum,* the ethyl acetate extract at 50 mg/ml concentration showed the highest zone of inhibition (*i.e.*, 12.17 mm) against *X. oryzae* while the same concentration of chloroform extract showed the highest zone of inhibition (*i.e.*, 6.84 mm) against *R. solanacearum*. Preliminary qualitative phytochemical studies of both the *Ocimum* species were done for the presence of phlobatannin, saponins, steroids, flavonoids, and tannins and for most of the cases, the results were found positive. HPLC fingerprint of the methanolic crude extracts of both the *Ocimum* species was done and *O. sanctum* showed the presence of about 38 numbers and *O. canum* about 34 numbers of metabolites. Thus the different solvent extracts of both the *Ocimum* species showed different antibacterial activities against *R. solanacearum* and *X. oryzae,* indicating their different sensitivities towards the metabolites present in the solvent extracts. Preliminary phytochemical studies of different solvent extracts and the HPLC fingerprint assay of methanolic extracts of both the *Ocimum* species reveal the presence of various phytoorganic constituents.

1. Introduction

The practices of using plant extract against microbial diseases have been known since ancient times worldwide. Due to the development of resistance in pathogens to antibiotics and synthetic drugs, there is an increased concern about the plant's system with antimicrobial properties.

In India, the use of tulsi (*Ocimum* spp.) plant in the traditional medical system is still in nascent stage, and it needs more scientific studies to establish its medicinal importance in the modern natural therapeutic practices. In Ayurveda, tulsi is described as the sources of tonic to revitalize not only body but also the mind and spirit. Tulsi contains numbers of important metabolites as well as some minor and trace elements those having solution to numbers of modern-day health related problems (Pandey *et al.*, 2007).

Today, multiple drug resistance in pathogenic microbes has been reported by many scientific findings which are mainly associated with the indiscriminate usage of commercial antimicrobial medicines, and it is more common in the case of infectious disease-causing microbes which frequently get exposed to the drugs in the name of treatment (Joshi *et al.*, 2009). Thus, scientists worldwide are searching for new alternatives which are having fewer side-effects and at the same time less harmful and persistent to the environment.

Tulsi is widely distributed in the tropical regions of Africa, Central and South America and Asia. The genus, *Ocimum* belongs to the family, Lamiaceae and comprises of 150 species (Dzoyem *et al.*, 2017). Among the best known species of *Ocimum*, the *O. sanctum*, which is aromatic in nature, is widely cultivated for its essential oil content, and other medicinal and perfumery purposes. In India, it is also grown for religious and some ceremonial occasions. *O. sanctum* is also reported to have antioxidant, antidiabetic, antiinflammatory, antimicrobial, antistress, anticancer and wound healing activities (Singh and Chaudhuri, 2018).

On the other hand, another species, *O. canum*, is reported to have antimicrobial, insecticidal, antidiabetic, anti-inflammatory, analgesic and anticancer activities (Selvi *et al.*, 2015). Essential oils derived from *O. canum* showed antimicrobial activity against Gram-negative bacteria (Bassole *et al.*, 2005). Extract of *O. canum* is also considerably used in Rwanda as the means of controlling insect pests during storage of food grains (Weaver *et al.*, 1991). The essential

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oil of *O. canum* contains active metabolites like chavicol, estragol eugenol and methyl eugenol those give faint yellow color and sharp odor. Unlike *O. basilicum*, *O. canum* contains more amount of camphor and no ethers (Xaasan *et al*., 1981). Essential oils extracted from *O. canum* possess insecticidal properties against adult *Anopheles funestus* (Belong *et al*., 2013). It is also known for its antioxidant and cytotoxic activities (Selvi *et al*., 2015).

The northeastern parts of India come under the Indo-Burma mega biodiversity hot-spot (Myers *et al*., 2000) and are endowed with various medicinal plants with a wide range of ecotypes. These biodiversity hotspots under the influence of the local environment might result in the evolution of secondary metabolites in the plants (Kessler and Kalske, 2018). Tulsi is considered as one of the ecotypes with numerous medicinal properties (Cohen, 2014). Although, there are many reports regarding the use of tulsi extract in medicinal practices for many diseases in humans, there is only a limited usage of tulsi extract against various plant diseases. In this study, the phytochemical analysis of two tulsi species, *O. sanctum* and *O. canum* was done, and their antimicrobial properties were evaluated against two important bacterial plant pathogens.

2. Materials and Methods

2.1 Collection of tulsi plants and preparation of plant extract

O. santum and *O. canum* leaf samples were collected during the summer at flowering stage from Ukhongsang region (24°38'12.9" N, 94°05'17.2" E) situated in the Thoubal district of Manipur, India. The aerial parts of *O. sanctum* as well as *O. canum* had been dried at room temperature (25-35°C) for 7-10 days and the ground them into fine powder using an electric grinder. Approximately, 35 g of powdered sample was extracted with 350 ml each of methanol, n-hexane, chloroform, and. ethyl acetate, separately. The extraction was continued for each organic solvent separately for 24 h until clear solvent was seen in the thimble. The homogenate thus prepared was filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 5000 g for 10-15 min. The resulting supernatant was sterilized through filtration with 0.22 mm filters (Millipore, Bedford, USA). The filtrate thus obtained was then concentrated using a rotary flash evaporator to a syrupy consistency at 40°C. The resulting syrupy material is then subjected to re-suspension in dimethyl sulfoxide (DMSO) solvent so that the final concentration attains 10 mg/10 ml for each extract. The stock concentration of each extract was stored at 4°C and used them during *in vitro* screening for their antimicrobial activities.

2.2 Qualitative analysis of phytochemicals

The qualitative analysis of various solvent extracts of both the tulsi species was done to identify important secondary metabolites having medicinal importance using standard methods described by Harborne (1973). The major constituents analyzed were phlobatannins, saponins, steroids, flavanoids and tannins.

2.3 Estimation of chlorophyll and carotenoids

The quantitative chlorophyll and carotenoids estimation was done following the methods as described by Arnon (1949). In this method, 1 g of cut fresh leaf samples was ground with 20 ml of 80% acetone. The crude extract thus prepared was centrifuged at 5000 rpm for 5-10 min. The resulting supernatant was collected and the process was repeated until the residues became colorless. The final volume of the supernatant was adjusted to 100 ml with 80% acetone in a volumetric flask. The absorbance (A) of the solution was measured at 480, 510, 645 and 663 nm in a spectrometer taking blank sample of acetone. Triplicate-readings were taken and the average value was considered for final calculation of chlorophyll and carotenoid amount. The following formulas were used to calculate the concentration of total chlorophyll and carotenoid:

- Total chlorophyll content (mg/gm of fresh tissue) = $\{20.2(A_{645}) + 8.02(A_{663})\} \times v/1000 \times w$
- Total carotenoid content (mg/gm of fresh tissue) = $\{7.6\ (A_{480}) - 1.49\ (A_{510})\} \times v/1000 \times w$

where,

A = absorbance at specific wavelengths

v = final volume of chlorophyll extract in 80 % acetone

w = weight of the fresh leave tissue extracted

2.4 Estimation of lipid content

Quantitative analysis of lipid content was made by following the method described by Bligh and Dyer (1959). 10 g of freshly collected leaves from both the Ocimum species was homogenized with chloroform and methanol (10:20, v/v) for 2 min. The repetition of the process was done for two times. The resulting suspension was then filtered using Whatman No.1 filter paper. The filtrate was collected in a cylinder. The mixture was allowed to stand for 10-15 min to develop two phases. The volume of chloroform layer developed at lower phase was recorded. The upper methanol-water layer was discarded, followed by removal of a minute volume of the chloroform layer to make it sure that the upper methanol-water layer gets completely removed. The remaining layer of chloroform was then transferred to a pre-weighed conical flask. The chloroform solvent was allowed to evaporate at 40-50°C in a water-bath, and then cooled. The resulting residue was dried in a vacuum desiccator over the phosphoric anhydride. The flask was weighed for a second time and the lipid content was dissolved in 5 ml of chloroform. This process was repeated again followed by weighing the flask for the third time. The following formula was used to calculate the lipid content in the plant sample:

% Total lipids = Total lipids (g)/ weight of the sample (g) × 100

2.5 HPLC analysis

The analysis of metabolites present in the methanol extract of *O. sanctum* and *O. canum* leaf extracts was done using UHPLC model Dionex (Thermo Fisher, USA) system. The HPLC quantitative separation was confirmed on a 5 µM C_{18} analytical column (4.6 × 250 mm) at wavelength 210 nm. The injected sample volume was kept at 20 µl, and the elution rate was fixed at 0.5 ml/min for about 30 min. The mobile phase comprised of water and acetonitrile (70%) in a 1:1 ratio.

2.6 Antibacterial assay

2.6.1 Plant pathogenic bacteria used in antibacterial assay

Two plant pathogenic bacteria, *X. oryzae* and *Ralstonia solanacearum* were obtained from the departmental microbial culture collecting centre of Department of Plant Pathology, Assam Agricultural University, Jorhat, and were used for the antimicrobial study. Both

the bacterial species were maintained on nutrient agar at 4°C. Before the susceptibility test, the bacterial species were freshly inoculated into nutrient broth and subjected for overnight incubation at 200 rpm and 37°C.

2.6.2 Adjustment of bacterial broth with 0.5 Mcfarland standard solutions

Prior to antimicrobial assay, the turbidity of the tested microorganisms was adjusted to 0.5 McFarland units that corresponds to a concentration of 1.5×10^8 colony forming units (CFU)/ml. To do the antibacterial susceptibility test using the agar well diffusion method, a cell suspension of tested bacteria equivalent to a 0.5 McFarland standard was used.

2.6.3 Antibacterial activity assay

Antibacterial assay of the different solvent extracts of *O. sanctum* and *O. canum* was done against *X. oryzae* and *R. solanaceurum*. The agar-well diffusion method as described by Parekh and Chanda (2007) was followed to do the antibacterial test. In this method, freshly grown broth cultures of tested bacterial pathogens were seeded over the nutrient agar plates using a sterile cotton swab. All total of five wells with 6 mm diameter were made at equidistance using cork borer on the nutrient agar plate. For the assay, 50 µl of five different concentrations of extract (10, 20, 30, 40 and 50 mg/ml), along with positive control (50 µg/ml streptomycin) and negative control (100% DMSO), were inoculated uniformly into the wells and incubated at 37°C overnight. Experiments were performed in triplicates. The mean inhibitory zone was calculated after 24 h with standard error.

3. Results

3.1 Qualitative screening of phytometabolites

In this present study, the presence of few phytometabolites having pharmaceutical importance, *viz*., phlobatannin, saponin, steroid, flavonoid and tannin, had been studied qualitatively in both Ocimum species. For this, the crude compounds present in the dried tissues were extracted in Soxhlet's apparatus in methanol, ethyl acetate, n-hexane and chloroform, separately. The crude extracts thus obtained were then assayed for the presence of the above said secondary metabolites. The results are presented in the Table 1. The presence or absence of a particular metabolite was marked as (+)ve or (-)ve, respectively.

Table 1: Screening of presence of phlobatannins, saponins, steroids, flavonoids, and tannins in *O. sanctum* and *O. canum*

Phyto-metabolites	*O. sanctum* extract				*O. canum* extract			
	Methanol	Ethyl acetate	n-hexane	Chloroform	Methanol	Ethyl acetate	n-hexane	Chloroform
Phlobatannins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Steroids	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Tannins	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve

From Table 1, it is clear that most of the phytometabolites studied, are present in both *Ocimum* species, at least anyone of the solvent extracts except saponin. The presence of saponin was found in all the solvent extracts of *O. canum*, but it was absent in the extracts *O. sanctum*. Again, it was observed that among the metabolites tested, phlobatannins and steroids were present in all the solvent extracts of both Ocimum species, except methanol extracts where Steroid was found absent in both species. In all the solvent extracts of both species, the presence of flavonoid was detected except in chloroform-extract of *O. canum* where it was nil. Tannin was detected in n-hexane and chloroform extracts of *O. sanctum* while in the case of *O. canum*, it was only present in methanol extract.

3.2 Quantitative estimation of phytometabolites

Along with the qualitative study of phlobatannin, saponin, steroid, flavonoid and tannin in both the *Ocimum* species, chlorophyll, carotenoids, and total lipid contents were also estimated quantitatively. For this, fresh leave tissues of *O. sanctum* and *O. canum* were used. 5 mg of fresh leaves was taken for the extraction and estimation of chlorophyll and carotenoids, while 10 g were taken for estimation of total lipid. Results are presented in Table 2.

Table 2: Chlorophyll, carotenoid, and lipid contents in *O. sanctum* and *O. canum*

Parameters	*O. sanctum*	*O. canum*
Total chlorophyll (mg/g)	1.65 ± 0.09	1.11 ± 0.02
Total carotenoids (mg/g)	0.50 ± 0.25	0.31 ± 0.01
Total lipids (mg/g)	1.23 ± 0.06	1.11 ± 0.01

From the results, it is observed that all three components were found higher in *O. sanctum* than in *O. canum*. The contents of chlorophyll, carotenoids and total lipids in *O. sanctum* were found 1.65 ± 0.09 mg/g, 0.50 ± 0.25 mg/g and 1.23%, respectively; whereas the same contents in *O. canum* were found 1.11 ± 0.02 mg/g, 0.31 ± 0.01 mg/g and 1.11%, respectively.

3.3 HPLC fingerprint of crude methanolic extract

For HPLC fingerprint assay, the powdered leaves samples of *O. sanctum* and *O. canum* were extracted in Soxhlet's extractor using methanol as the solvent. The HPLC chromatograms are presented in the Figure 1, and the retention time (RT), height and relative area in the chromatograms obtained are presented in the Table 3.

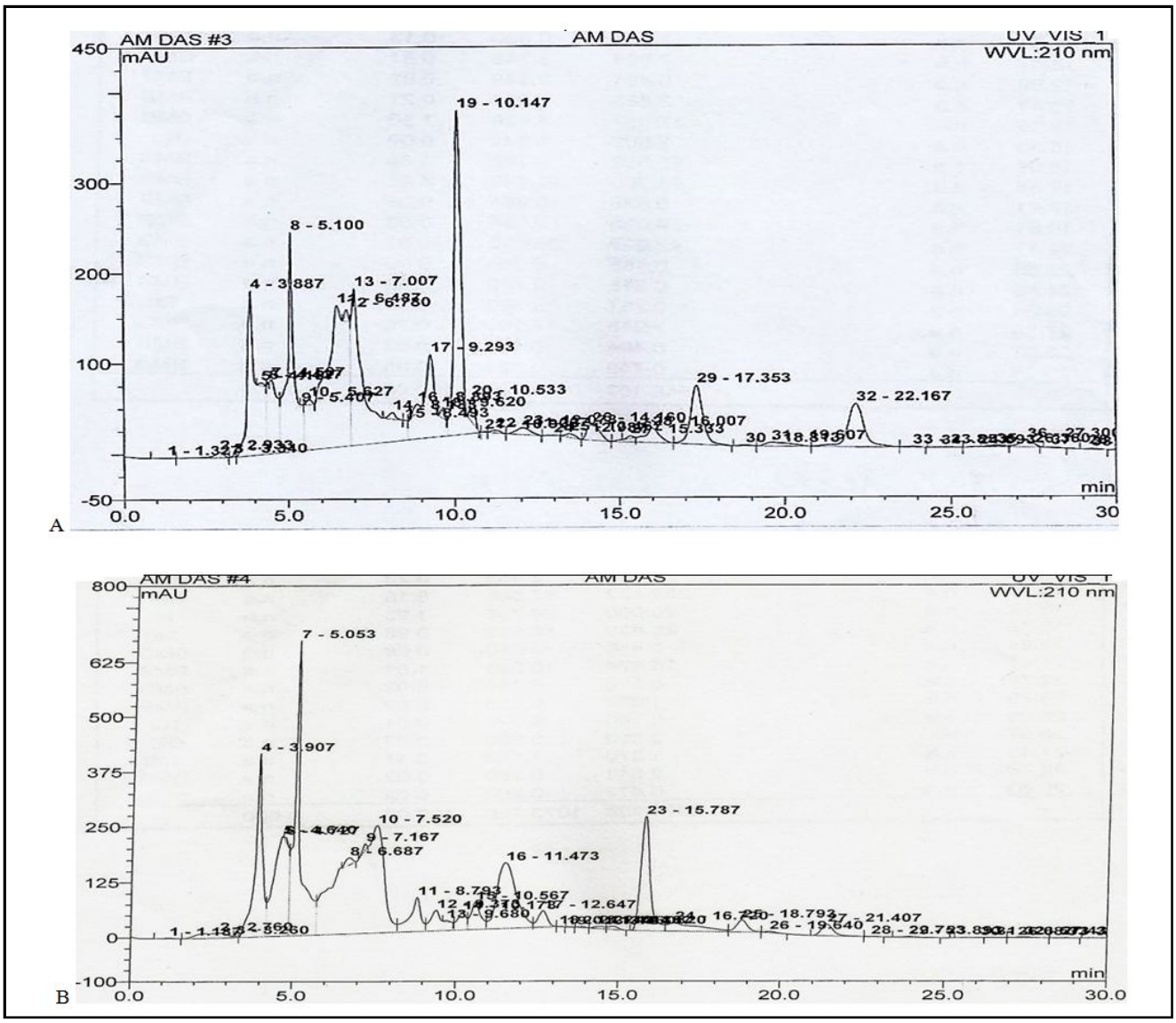

Figure 1: HPLC chromatogram of crude methanolic extract, A. *O. sanctum*, B. *O. canum.*

Table 3: Retention time, height and relative area of peak representing individual metabolite from HPLC chromatogram of crude methanolic extracts of *O. sanctum* and *O. canum*

S.No.	*O. sanctum*			*O. canum*		
	Retention time (min)	Height (mAU)	Relative area (%)	Retention time (min)	Height (mAU)	Relative area (%)
1	1.33	0.214	0.01	1.19	1.473	0.06
2	2.93	5.916	0.36	2.76	10.356	1.05
3	3.34	1.915	0.03	3.26	1.142	0.01
4	3.89	181.742	10.35	3.91	415.117	9.66
5	4.19	2.955	0.09	4.64	225.347	10.86
6	4.43	1.479	0.01	4.73	7.509	0.08
7	4.51	81.764	4.66	5.05	670.462	14.17
8	5.10	242.300	10.15	6.69	15.293	0.36
9	5.41	0.209	0.00	7.17	12.946	0.18

10	5.63	6.185	0.17	7.52	242.330	31.85
11	6.49	156.005	21.87	8.79	77.751	2.86
12	6.78	12.748	0.26	9.37	46.752	2.09
13	7.01	172.822	14.16	9.68	2.737	0.04
14	8.13	7.364	0.26	10.17	41.252	1.16
15	8.49	0.346	0.01	10.57	62.310	2.01
16	8.89	7.466	0.27	11.47	148.338	9.47
17	9.29	91.330	7.32	12.65	36.728	1.14
18	9.62	9.008	0.19	13.14	0.315	0.01
19	10.15	358.866	14.79	13.37	0.003	0.00
20	10.53	27.741	0.74	13.86	2.051	0.04
21	10.90	0.508	0.01	14.39	4.748	0.17
22	11.23	2.597	0.13	14.82	7.030	0.23
23	12.07	7.624	0.61	15.79	258.432	8.16
24	12.99	0.481	0.02	16.72	20.000	1.93
25	13.47	3.885	0.21	18.79	26.630	0.98
26	14.16	17.057	1.26	19.64	2.446	0.08
27	15.33	2.608	0.09	21.41	26.174	1.01
28	16.01	15.552	1.59	22.75	0.510	0.02
29	17.35	64.763	4.48	23.89	1.664	0.09
30	18.81	0.938	0.06	26.09	0.100	0.01
31	19.61	4.033	0.58	26.57	0.893	0.08
32	22.17	47.629	4.33	27.43	1.870	0.11
33	23.85	0.785	0.05	28.76	0.541	0.02
34	24.69	0.315	0.03	29.63	0.473	0.02
35	26.36	0.761	0.06	-	-	-
36	27.30	7.013	0.70	-	-	-
37	28.01	0.494	0.03	-	-	-
38	29.25	0.749	0.05	-	-	-

Both Figure 1 and Table 3 show that *O. sanctum* contains about 38 nos. and *O. canum* contains about 34 nos. of metabolites in their methanolic extracts in terms of distinct peaks in the chromatograms. Having the same RT for both *Ocimum* species indicates the probability of presence of same metabolites and a completely different RT indicates the probability of having different metabolites. Minor or non-considerable differences in RT may be considered instrumental errors. The differences in the relative area of peaks with the same RT indicate the presence of same component with different quantities.

3.4 Antibacterial activities of tulsi extracts against plant pathogens

The antibacterial activity of both the *Ocimum* species was evaluated against two bacterial plant pathogens of agricultural importance, *viz., X. oryzae* and *R. solanacearum* following the agar well diffusion method. For this test, five different concentrations (10, 20, 30, 40 and 50 mg/µl) of each solvent extract, *i.e.*, methanol, ethyl acetate, n-hexane and chloroform, were tested with three replications. After drying of solvent in each extract, DMSO was added to re-dissolve the extracted metabolites and the resulting DMSO-dissolved sample was tested for antibacterial activity. Therefore, DMSO was taken as the negative control. The antibacterial, streptomycin (50 µg/ml), was taken as a positive control. In all the cases, 50 µl was taken as the test volume. After 24 h of incubation, the zone of clearance or zone of inhibition was observed. The presence or absence of the zone of inhibition indicates susceptibility or resistance of the particular bacterial pathogen against the metabolite(s) present in the specific solvent extract. Again, the size of the inhibition zone was also considered as the indication of intensity of susceptibility or resistance.

3.4.1 Antibacterial activities of tulsi extracts against *X. oryzae* and *R. solancearum*

The antibacterial activities of *O. sanctum* and *O. canum* against *X. oryzae* and *R. solancearum* are presented in Tables 4 and Table 5, respectively. Both the Ocimum species showed antibacterial activities against the *X. oryzae* and *R. solancearum* but with varying degree of effectiveness.

Table 4: Antibacterial activity of *O. sanctum* and *O. canum* against *X. oryzae*

Plant	Extract of the solvents used	Zone of inhibition (mm)					
		10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	* (+) ve control 0.05 mg/ml
O. sanctum	n-hexane	5.5 ± 0.25	5.67 ± 0.22	6.5 ± 0.22	7.33 ± 0.25	7.33 ± 0.12	17.53 ± 0.28
	Ethyl Acetate	2.5 ± 0.34	5 ± 0.12	5.83 ± 0.25	6.5 ± 0.12	8 ± 0.59	17.53 ± 0.28
	Chloroform	4.5 ± 0.22	4.66 ± 0.25	5.83 ± 0.12	7.66 ± 0.12	9.66 ± 0.12	17.53 ± 0.28
	Methanol	3.83 ± 0.22	4.16 ± 0.22	4.16 ± 0.12	4.66 ± 0.22	6.16 ± 0.46	17.53 ± 0.28
O. canum	n-hexane	4.33 ± 0.25	4.83 ± 0.12	6.33 ± 0.25	7.5 ± 0.38	8.83 ± 0.12	18 ± 0.45
	Ethyl Acetate	5.9 ± 0.12	8.33 ± 0.25	10.67 ± 0.25	11.83 ± 0.12	12.17 ± 0.12	18 ± 0.45
	Chloroform	3.88 ± 0.12	5.83 ± 0.12	6 ± 0.67	6.83 ± 0.12	6.83 ± 0.12	18 ± 0.45
	Methanol	3.9 ± 0.12	4.5 ± 0.22	5.5 ± 0.22	5.67 ± 0.12	8.83 ± 0.12	18 ± 0.45

*(+) ve control, streptomycin.
(-) ve control is not shown in the table as it did not show any zone of clearance.

Table 5: Antibacterial activity of *O. sanctum* and *O. canum* against *R. solancearum*

Plant	Extract of the solvents used	Zone of inhibition (mm)					
		10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	*(+)ve control 0.05 mg/ml
O. sanctum	n-Hexane	2.66 ± 0.52	3.5 ± 1.26	4 ± 1.71	4.66 ± 2.4	5.67 ± 3.0	17.3 ± 0.26
	Ethyl acetate	1.16 ± 0.12	3.16 ± 0.25	4.33 ± 0.34	5.33 ± 0.12	5.5 ± 0.12	17.3 ± 0.26
	Chloroform	1 ± 0.12	1.16 ± 0.38	3.5 ± 0.34	3.66 ± 0.46	5.33 ± 0.12	17.3 ± 0.26
	Methanol	3.5 ± 0.12	3.83 ± 0.46	4.5 ± 0.22	6.5 ± 0.59	7.16 ± 0.12	17.3 ± 0.26
O. canum	n-Hexane	1.17 ± 0.52	2.83 ± 1.26	3.84 ± 1.71	5.5 ± 2.45	6.76 ± 3.05	17.03 ± 0.15
	Ethyl Acetate	0.67 ± 0.12	2.33 ± 0.25	2.84 ± 0.34	3.33 ± 0.12	3.33 ± 0.12	17.03 ± 0.15
	Chloroform	1.66 ± 0.12	6 ± 0.38	5.84 ± 0.34	5.84 ± 0.46	6.84 ± 0.12	17.03 ± 0.15
	Methanol	0.83 ± 0.12	1.34 ± 0.46	2.5 ± 0.22	3 ± 0.59	4.17 ± 0.12	17.03 ± 0.15

*(+) ve control, streptomycin.
(-) ve control is not shown in the table as it did not show any zone of clearance.

However, from the Tables 4 and 5, it is clear that irrespective of the solvent systems used, with the increase in concentrations (up to 50 mg/ml) of the solvent extracts, the size of the zone of inhibition increased for both *Ocimum* species indicating more effective inhibition at higher concentration.

From the Table 4, it was observed that, the ethyl acetate extracts of *O. canum* at a concentration of 5 mg/ml showed the highest zone of inhibition (12.17 ± 0.12 mm) against *X. oryzae*, followed by methanol/ n-hexane extract (8.83 ± 0.12 mm) and chloroform extracts (6.8 ± 0.12 mm). But, in the case of *O. sanctum,* the chloroform extract at 5 mg/ml concentration showed the highest zone of inhibition (9.66 ± 0.12 mm) against the same plant pathogenic bacteria followed by ethyl acetate extract (8 ± 0.59 mm), n-hexane extract (7.33 ± 0.12 mm) and methanol extract (6.16 ± 0.46 mm).

Again, from the Table 5, it was observed that, the methanol extract of *O. sanctum* at a concentration of 5 mg/ml showed the highest zone of inhibition (7.16 ± 0.12 mm) against *R. solancearum*, followed by n-hexane extract (5.67 ± 0.3 mm), ethyl acetate extract (5.5 ± 0.12 mm) and chloroform extract (5.33 ± 0.12 mm). But, in the case of *O. canum,* the chloroform extract at 5 mg/ml concentration showed the highest zone of inhibition (6.84 ± 0.12 mm) against the same pathogenic bacteria followed by n-hexane extract (6.76 ± 3.05 mm), methanol extract (4.17 ± 0.12 mm) and ethyl acetate extract (3.33 ± 0.12 mm).

It was also observed that the zone of inhibition in the case of positive control (streptomycin at 0.05 mg/ml) was considerably higher (approx.17.3 mm for both *X. oryzae* and *R. solancearum*) as compare to all other cases irrespective of solvent systems as well as concentrations of plant extract used for both Ocimum species. On the other hand, in the case of negative control no zone of inhibition was observed, as expected.

The results of the relevant assays, as described in Table 4 and 5, are presented in Figures 2a and 2b and Figures 3a and 3b (only at 50 mg/ml concentration is shown), respectively.

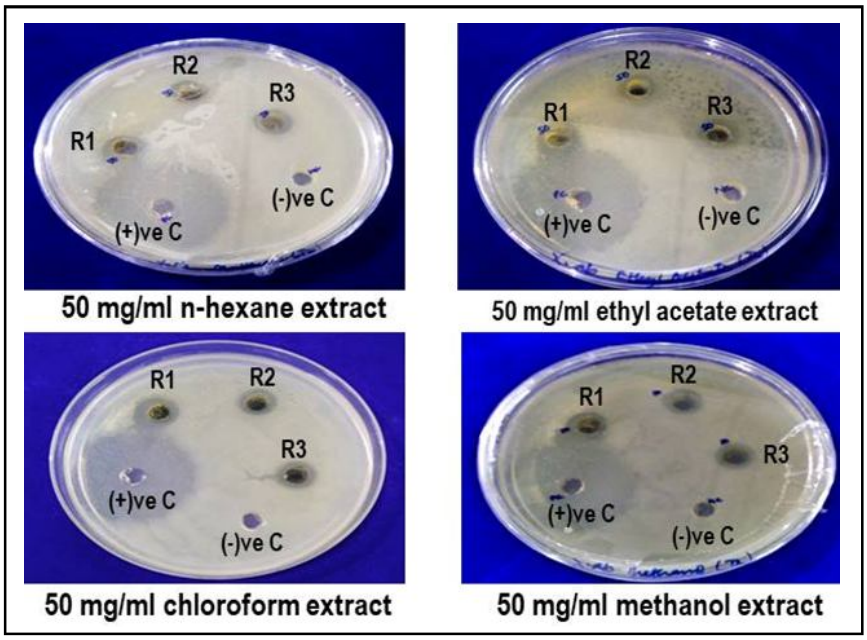

Figure 2a: Inhibition zone of *O. sanctum* against *X. oryzae*; R1, R2, R3 are 3 replications; +ve C indicates positive control and –ve C indicates negative control.

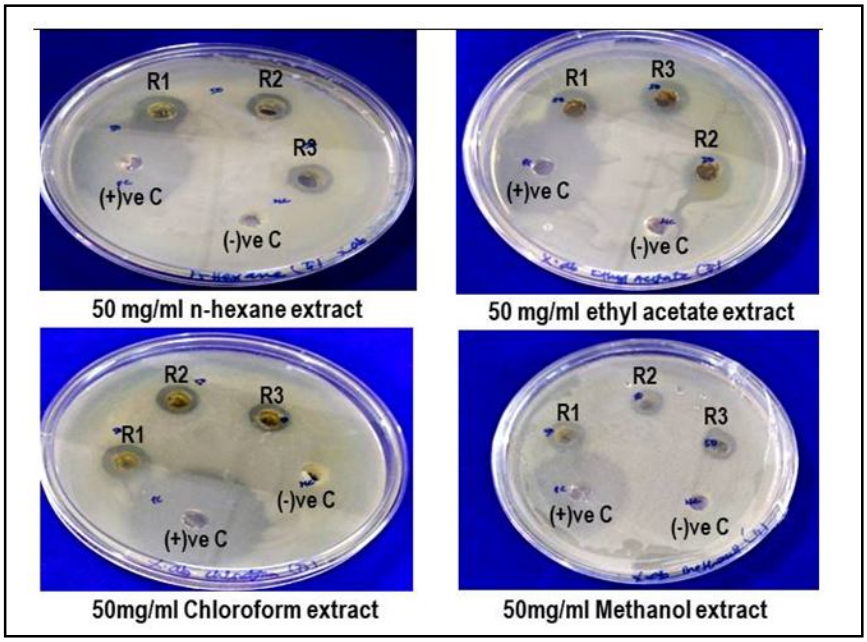

Figure 2b: Inhibition zone of *O. canum* against *X. oryzae*; R1, R2, R3 are 3 replications; +ve C indicates positive control and –ve C indicates negative control.

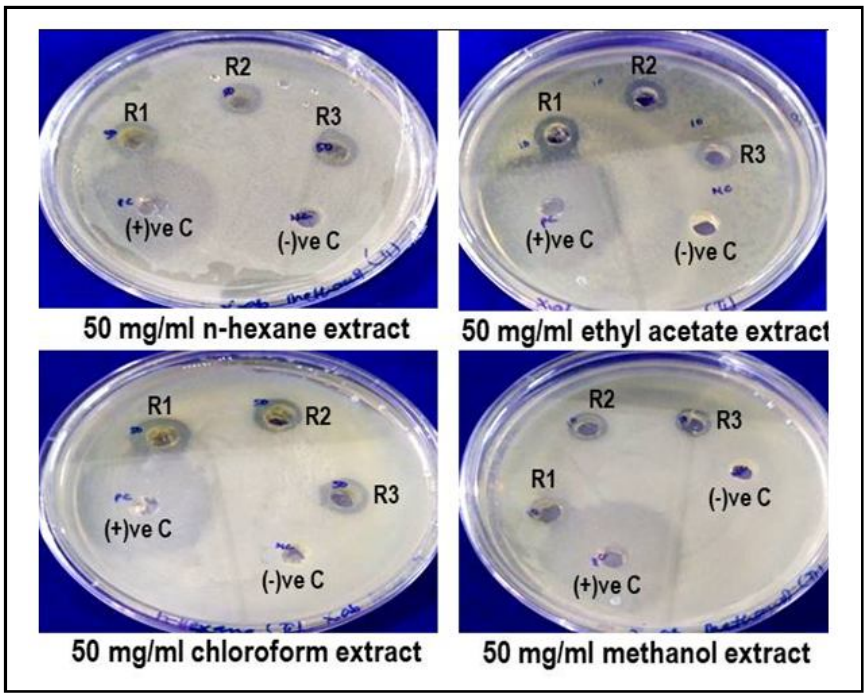

Figure 3a: Inhibition zone of *O. sanctum* against *R. solanacearum*; R1, R2, R3 are 3 replications; +ve C indicates positive control and –ve C indicates negative control.

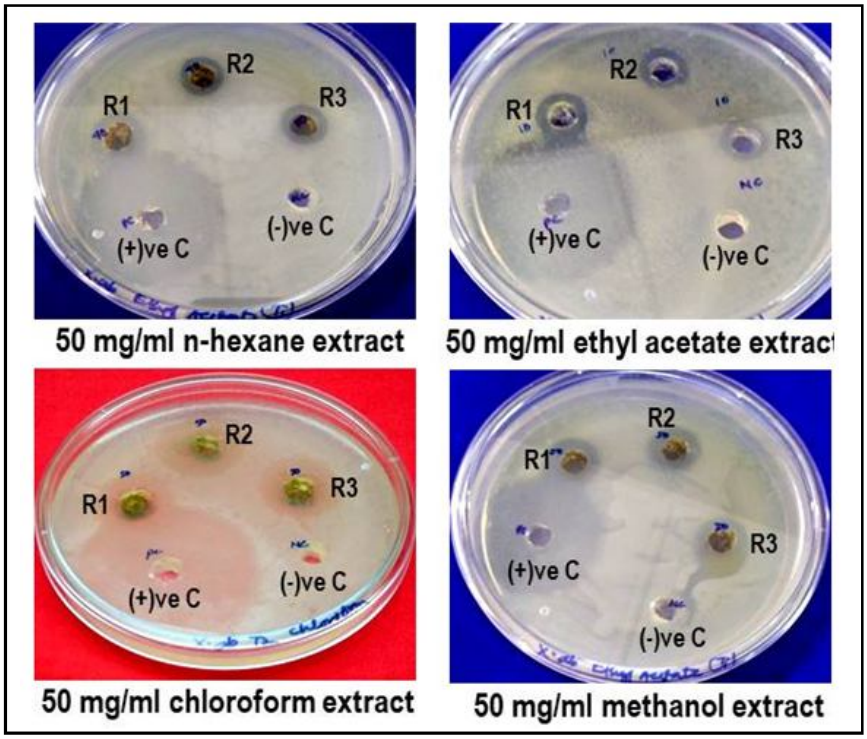

Figure 3b: Inhibition zone of *O. canum* against *R. solanacearum*; R1, R2, R3 are 3 replications; +ve C indicates positive control and –ve C indicates negative control.

4. Discussion

In the present study, the presence of few phytometabolites having pharmaceutical importance, *viz*., phlobatannin, saponin, steroid, flavonoid and tannin had been studied qualitatively in both *Ocimum* species. The qualitative phytochemical screening indicated the presence of important active constituents of therapeutic importance

and revealed that both *Ocimum* species have almost similar phytochemical constitutions. In the leaf extracts of methanol, ethyl acetate, n-hexane and chloroform of both *Ocimum* species showed the presence of major secondary metabolites, *viz*., tannins, phlobatannins, steroids, flavanoids and saponins, though their presence or absence was varied either the studied plant species-wise or used solvent-wise (Table 1). The plant species-wise variation is mostly while the solvent-wise variation is determined by the solubility of the particular compound in the solvent systems used for the purpose. The solubility of a compound in a particular solvent depends upon the nature of both the compound and the used solvent. Generally, polar-polar or nonpolar-nonpolar attraction is more than the polar-nonpolar attraction which ultimately determines the solubility of a polar or non-polar compound in a polar or non-polar solvent. Panche *et al*. (2016) also reported the presence of flavonoids in *O. canum* extract and he proposed that due to presence of flavonoid, it may possess antiseptic, anticancer, and anti-inflammatory properties. Based on some earlier studies on the medicinal properties of plants, the anti-inflammatry and analgesics effects of *O. canum* can be attributed to the presence of secondary metabolites such as tannins, steroids, phlobatannins and saponins (Ayinde *et al*., 2007; Hasan *et al*., 2014). Flavanoids present that presented in the leaf and stem extracts of *O. sanctum* were reported to have free radical scavenging activity and exhibited radiation-induced protection suggesting its radio-protective effect and antioxidant properties (Kelm *et al*., 2000). Sharma *et al*. (2011) also reported the presence of various pharmacologically active compounds such as alkaloids, tannins, saponins, flavonoids, and amino acids in *O. gratissimum, O. sanctum*, and *O. canum*. Naik *et al*. (2015) conducted phytochemical screening of *O. tenuflorum* and reported the presence of medicinally active constituents like tannins, alkaloids, terpenoids, steroids, flavnoids, phlobatannins, and glycosides in the leaf extracts while they reported the absence of saponin in the same samples. Sharma *et al*. (2021) also reported presence of flavonoids and alkaloids qualitatively both in methanol and chloroform extracts in *Tamarindus indica* L.

Along with the qualitative study of phlobatannin, saponin, steroid, flavonoid and tannin in the *Ocimum* species, chlorophyll, carotenoids and total lipid contents were estimated quantitatively. The variation in chlorophyll, carotenoids and total lipid content in these two *Ocimum* species might be attributed to the species level genetic variation. Ghosh *et al*. (2018), while studying photosynthetic pigments in 21 medicinal plants including *O. sanctum*, observed that the chlorophyll and carotenoid contents of *O. sanctum* were 1.201 ± 0.012 and 0.676 ± 0.013 mg/g of tissue, respectively, which is comparable with the results obtained in the present studies for the same species. Chlorophyll and carotenoid are two important photosynthetic pigments found in algae, cyanobacteria and higher plants (Grabowski *et al*., 2001) and provide protection in the prevention of various oxidative-stress related diseases such as cancer, cardiovascular diseases and several chronic diseases (Tapiero *et al*., 2004). Functional lipids from plants or phytosterols are biologically active molecules that decrease blood cholesterol and prevent cardiovascular diseases (Lichtenstein and Deckelbaum, 2001). Ezeonu and Ejikeme (2016) estimated phytochemicals including total lipid content in 24 softwood plants and observed that the lipid content varied from 1.2 to 8.0%. The total lipid content in the present study for both Ocimum species was within their reported range (1.11-1.23%).

For HPLC fingerprinting assay, the powdered leaves samples of *O. sanctum* and *O. canum* were extracted in Soxhlet's extractor using methanol as the solvent (Figure 1, Table 3). In the present study, the RT ranged between 1.33 to 29.25 min for *O. sanctum* and 1.19 to 29.63 min for *O. canum*. Das *et al*. (2017) determined the chromatographic profiles of two varieties of *O. sanctum* leaf extract. The HPLC chromatograms revealed the presence of 11 and 9 compounds in dark-pigmented and bright-pigmented variety of *O. sanctum*, and the RT ranged from 1.77- 5.29 min and 1.92-6.27 min, respectively. In another study, Deo *et al*. (2011) performed HPLC analysis of metabolic extracts of *O. sanctum* and *O. kilimandsacharicum* which showed major peaks at RT of 2.63, 2.85, 3.03, 2.61and 7.37 min. Rahman *et al*. (2021) did HPLC fingerprint assay of methanolic extracts of *O. sanctum* and *O. gratissimum*, and found 171 and 145 peaks, respectively.

The antibacterial activities of *O. sanctum* and *O. canum* against *X. oryzae* and *R. solancearum* are presented in Tables 4 and Table 5, respectively. The antibacterial activities of plant extracts have also been reported by several authors (Adebolu and Oladimeji, 2005; Goel and Kaushik, 2011; Seshadri, 2021; Krishnarao and Rajeswari, 2023). In the present study, the antibacterial activities of the different organic extracts of the two *Ocimum* species against *X. oryzae* and *R. solanacearum* varied greatly which might be associated with the variation in presence of active molecules and their concentrations having antibacterial properties in different solvent extracts. Murthy *et al*. (2014) conducted antibacterial studies of *O. sanctum* extract on *R. solanacearum* and they observed that the most active extract was methanol extract with the inhibition zones in the range of 9 to 18 mm diameter. Adebolu and Oladimeji (2005) evaluated the antibacterial activity of leaf extracts of *O. sanctum* against diarrhea-causing bacteria (*Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *S. typhimurium*) and found that only the steam distillation extracts showed inhibitory effects against the pathogens. Nascimento *et al*. (2011) studied on the composition of essential oils of *O. canum* and *O. selloi* and their antimicrobial activity. They found that all extracted oils from the aerial parts both the *Ocimum* species showed antibacterial activity against the Gram-positive bacterial species, *S. aureus*. Moreover, the oil extract from the *O. canum* also showed antibacterial activity against *E. coli* which is Gram-negative one. Rahman *et al*. (2021) studied the effect of different solvent extracts, *viz*., methanol, petrolium ether, n-hexane and water of *O. sanctum* and *O. gratissimum* against two bacterial species, *E. coli* and *Agrobactrium tumefaciens*, and found that among the different solvent extracts used, the methanolic extract was the most effective one against both bacterial species as it showed the largest zone of inhibition.

The research presented in this paper is comprises of fundamental study of a masters student. The research could be further extended to study the antimicrobial activities of tulsi against more numbers of both animals and plant pathogenic microbes. Specific molecules present in both the tulsi species can be isolated, purified and screened for their individual pharmaceutical activities which will lead to develop effective drug formulation against specific pathogens.

5. Conclusion

The results of the present study suggest that, the different solvent extracts, *viz*., methanol, n-hexane, chloroform and ethyl acetate of the Ocimum species, *O. sanctum,* and *O. canum*, possess compounds

with antimicrobial properties. The antimicrobial activity against the tested bacterial pathogens, *R. solanacearum,* and *X. oryzae* was found to be increased in all testing systems with increasing concentrations of solvent extracts. In the qualitative phytochemical screening, the secondary metabolites, *viz*., phlobatannins, saponins, steroid, flavonoids and tannins, were found present in all the solvent extracts of both the *Ocimum* species except saponin which was absent in all the solvent extracts of *O. sanctum*. HPLC fingerprint assay of methanolic extracts of *O. sanctum* and *O. canum* also revealed the presence of various phytoorganic constituents of pharmaceutical importance in both *Ocimum* species. Antimicrobial assay of the crude extracts using different solvent systems showed varying of sensitivity against *R. solanacearum* and *X. oryzae*. The results obtained justify the use of these two *Ocimum* species as antibacterial. However, it is quintessential to study the bioactivity of different solvent extracts of both the *Ocimum* species to develop new antibacterial formulation.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article..

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