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Phytochemical screening, antibacterial activity and antioxidant activity of *Ocimum sanctum* leaf extract

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Abstract
Ocimum sanctum has a rich and fanciful history known since the Vedic age for its immense curative and multi-purpose utility. The phytochemical results show the presence of Amino Acid, Carbohydrate, Phenol and Terpenoids in all the four solvent extracts of the *O. santum*. Except Aqueous extract of the plant all other extracts show the positive results for alkaloids, Coumarin, Flavonoids and Glycoside. Saponin is present in the benzene and water solvent extracts of *O. sanctum*. The four solvent extracts are showing the absence of following phytochemical's such as Tannins, Phlobatanins, Quinones and Anthocyanin. The higher concentration Acetone extract, Benzene extract, Methanol extract and Water extract of the *O. sanctum* show the activity against the tested pathogens by the following order from the highest zone of inhibition *P. aeruginosa* (8.5 mm), *S. typhi* (5 mm) and *E. coli* (3.5 mm) for Acetone extract, *E. coli* (4 mm) only for Benzene extract, whereas *P. aeruginosa* (14 mm), *S. typhi* (8 mm) and *E. coli* (7 mm) for Methanol extract and there is no activity for the water extract for all the three pathogens were observed. The antioxidant activity of the *O. sanctum* solvent extracts was high to Methanol followed by Benzene, Acetone, Water extracts. The results of the study indicate that the *Ocimum sanctum* possesses phyto-constituents having antibacterial activity thus it can be utilized as a natural plant based antimicrobials.

Keywords: Tulsi, *O. sanctum*, *P. aeruginosa*, *S. typhi*, *E. coli*, phytochemical, antioxidant

1. Introduction
India is called as the botanical garden of the world as it is the largest producer of medicinal herbs. In rural India, 70 per cent of the population depends on the traditional type of medicine, the Ayurveda. Many forms of alternative medicines are available for those who do not want conventional medicine or who cannot be helped by conventional medicine. *Ocimum sanctum* (synonym *Ocimum tenuiflorum*), commonly known as holy basil, tulasi (sometimes spelled thulasi) or tulsi, is an aromatic perennial plant in the family Lamiaceae. An erect much branched softly pubescent undershrub, 30-60 cm high with red or purple sub quadrangular branches; leaves simple, opposite, elliptic, oblong, obtuse or acute, entire, serrate or dentate, pubescent on both sides, minutely gland dotted, petioles slender, hairy; flowers small, purplish, in terminal thyrsoid panicles; calyx purplish, 2-lipped, pubescent, upper lip orbicular, reflexed, lower lip 4-lobed; corolla 2-lipped; stamens 4, didynamous, filaments purple, anthers yellow; nutlets ellipsoid, smooth, mucilaginous when wetted. The leaves are acrid, thermogenic, aromatic, antibacterial, insecticidal, antiviral, appetizing, and deodorant. They are useful in cough, bronchitis, catarrh, halitosis, bacterial and viral infections, foul ulcers. The aim of the present research work is to evaluate the biological activities of organic and aqueous extracts of *Osmium sanctum* species against bacteria, so to evaluate the phytochemical contents and establish the antioxidant potentials.

2. Materials and Methods
2.1 Collection of Plants
The leaves of *O. sanctum* were collected from Thimampettai village, Vaniyambadi, in October 2018 (Fig 1). The leaves were identified and authenticated by Dr. N.P.M. Mohamed Tariq, Assistant professor of Biotechnology, Islamiah College (Autonomous), Vaniyambadi. After identification, the plant material was processed for extraction.

Fig 1: Collection of *Ocimum sanctum* plant leaf

2.2 Preparation of Plant Extract

The leaves of O. *sanctum* were thoroughly cleaned with water to remove dust particles and shade – dried at room temperature and reduced to coarse powder using a mechanical mixer**.** The powder was subjected to extraction by maceration using various solvents like Acetone, Benzene, Methanol and Water to obtain their respective extracts. 5gm of the powder in 100ml solvent (Acetone, Benzene, Methanol, Water) was added and stirred occasionally in orbital shaker**.** The mixture was filtered on the 2nd day and the solvent was evaporated at room temperature for 18-24 hours to obtain a solid mass, which are stored in refrigerator (4ºC) for further use.

2.3 Phytochemical Screening

2.3.1 Alkaloids

a) Mayer's test

1 ml of extract and 1 ml of Mayer's reagent are added. Appearance of white creamy precipitate indicate the presence of Alkaloids. (Rimjhim Sheel *et al*., 2014) [13].

b) Wagner's test

1 ml of extract and 1ml of Wagner's reagent are added. Appearance of reddish-brown precipitate indicate the presence of Alkaloids. (Joseph BS *et al*., 2013) [5].

2.3.2 Amino Acid

Xanthoprotein test

1 ml of extract and 1 ml of Conc. Nitric Acid are added (white precipitate is formed) it is heated for 2-3 minutes and cooled. Then 1 ml of 20% of NaOH is added. Appearance of orange colour indicates the presence of Aromatic amino acid. (Prashant Tiwari *et al*., 2011) [11].

2.3.3 Carbohydrate

Molish's test

2 ml of extract and 2 ml of molish's reagent and 2 ml of conc.H_2SO_4 are added. Appearance of reddish ring indicates the presence of Carbohydrate. (Joseph BS *et al*., 2013) [5].

2.3.4 Phenol

a) $FeCl_3$ test

1 ml of the extract and 1 ml of 5% ferric chloride are added. Appearance of dark green colour / reddish brown / blue / violet / purple indicates the presence of phenol. (Ashok Kumar *et al*., 2012) [1].

b) Potassium dichromate test

2 ml of extract and 1 ml of 10% of potassium dichromate are added. Appearance of red colour indicates the presence of phenol. (Rajalakshmy M.R *et al* 2011) [12].

2.3.5 Flavonoids

Alkaline reagent test

1 ml of the extract and 1 ml of the 10% of sodium hydroxide are added. Appearance of yellow fluorescence indicates the presence of flavonoids. (Ashok Kumar *et al*., 2012) [1].

2.3.6 Tannins

FeCl3 test

2 ml of the extract and 2 ml of the 5% ferric chloride are added. Appearance of green colour indicates the presence of Tannins (C Yogeshwari *et al*., 2017) [18].

2.3.7 Saponin

Foam test

2 ml of the extract and 2 ml of the Dis.H_2O are added and shaken vigorously. Formation of stable foam indicates the presence of Saponins (Rimjhim Sheel *et al*., 2014) [13].

2.3.8 Terpenoids

a) Salkowskis test

1 ml of the extract and 2 ml of the chloroform and 3 ml of the conc.H2SO4 are added. Appearance of yellow/reddish brown colour indicates the presence of Terpenoid's (Ashok Kumar *et al*., 2012) [1].

b) Libermann-Burchard's test

2 ml of the extract and 2 ml of the chloroform and 2 ml of the acetic acid, 1 ml of the conc.H2SO4 are added. Appearance of blue green colour/reddish ring indicates the presence of Terpenoid's (Ashok Kumar *et al*., 2012) [1].

2.3.9 Phlobatanins

1% Hydrochloric acid test

2 ml of the extract and 2 ml of the 1% HCL test are added and heated in boiling water bath. Appearance of red colour indicates the presence of Phlobatanins (N. Lata *et al*., 2010) [7].

2.3.10 Quinones

Hydrochloric Acid test

1 ml of the extract and 1 ml of the conc. HCL are added. Appearance of yellow colour indicates the presence of Quinone's (R Dhivya *et al*., 2013) [4].

2.3.11 Coumarin

Sodium hydroxide test

1 ml of the extract and 1 ml of 10% sodium hydroxide are added. Appearance of yellow colour indicates the presence of Coumerin (C Yogeshwari *et al*., 2017) [18].

2.3.12 Glycoside

Kellar-Kilianis test

2 ml of the extract and 2 ml of the glacial acetic acid and few drops of the 5% $FeCl_3$ and conc.H_2SO_4 are added. Appearance of reddish brown/blue green colour indicates the presence of Glycoside's (Joseph BS *et al*., 2013) [5].

2.3.13 Anthocyanin

Sulphuric acid test

1 ml of the extract and 1 ml of concentrated sulphuric acid are added. Appearance of yellowish orange colour indicates the presence of Anthocyanin (Seema Firdouse *et al*., 2011) [14].

2.4 Antibacterial Activity

The Disc Diffusion Method for Antimicrobial Susceptibility testing was used to evaluate the presence of Antibacterial Activities of the different solvent extracts of *O. sanctum*. *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* used for the testing are collected from Department of Microbiology, Shanmuga Industries of Arts and Science College, Tiruvannamalai. The bacteria are cultured overnight at 37°C in Nutrient broth. The sterile Muller Hinton Agar (Hi-media) plates were prepared. The 100 mg/ml and 200 mg/ml of the Acetone, Benzene, Methanol, Water solvent extracts were prepared by using the respective solvents. Then 200µl of the bacterial suspension was introduced into the sterile plates and spreading the bacteria using L-Rod to get an even culture all over the plates. The 6 mm Discs was prepared from Whatman No.1 filter paper and it also autoclaved. A plate comprises of four discs, one is positive control, one is negative control and two for the two different concentrations (100, 200 mg/ml) of the same plant extract. Ampicillin is used as positive control and respective solvents in which the sample is dissolved was used as negative control. The discs are impregnated with positive control, negative control, two different concentration of the plant extract were prepared and placed on the prepared Muller Hinton Agar plates. Then all the plates are kept for incubation at 37°C for 24 hours.

2.5 Antioxidant Activity

The Antioxidant activity of the solvents extracts was performed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. A solution of 0.135mM DPPH was freshly prepared by dissolving 4 mg of DPPH in 100 ml of Methanol. The 2 ml of the different concentrations of extracts (20 µg, 40 µg, 60 µg, 80 µg, 100 µg, 150 µg, 200 µg, 250 µg/ml of Acetone, Benzene, Methanol, Water solvents) is taken and 2 ml of the DPPH solution (0.135mM) was added. The control sample was prepared by 2 ml of DPPH solution alone without sample mixed with 2 ml of methanol**.** Then all the test tubes were incubated in dark room for 30 minutes at room temperature. After incubation is completed the changes in the absorbance was measured at 517 nm in UV-Spectrophotometer. Methanol alone used as blank to set zero in the UV-Spectrophotometer. The antioxidant activity was expressed in inhibition percentage by using following formula,

$$\text{Inhibition percentage} \frac{[\text{Control O.D} - \text{Sample O.D}]}{\text{Control O.D}} \text{X } 100$$

3. Result and Discussion

3.1 Phytochemical screening

The phytochemical analysis of the various solvents extract is tested for 13 phytochemicals. The procedure for the phytochemical analysis is carried out using standardized protocols (Jyotsana sharma *et al*., 2017) [15]. The results shows presence of Amino Acid, Carbohydrate, Phenol and Terpenoids in all the four solvent extracts of the *O. santum*. Except Aqueous extract of the plant all other extracts shows the positive results for alkaloids, Coumarin, Flavonoids and Glycoside. The saponin is present in the benzene and water solvent extracts of *O. sanctum*. The four solvent extracts are showing negative result for the following phytochemical's such as Tannins, Phlobatanins, Quinones and Anthocyanin (Table. 1). The present findings indicate that *Ocimum* possesses compounds with antimicrobial properties against pathogenic microorganisms as reported by Prasad M.P *et al* (2012) [10].

3.2 Antibacterial activity

The Disc Diffusion Method (Ashraf Mostafa *et al*., 2017) [2] for Antimicrobial Susceptibility testing was used to evaluate the presence of Antibacterial Activities of the different solvent extracts of the *O. sanctum*. *E. coli, P. aeruginosa, S. typhi* are used for the testing. Bacteria are cultured overnight at 37°C in Nutrient broth. The sterile Muller Hinton Agar (Hi-media) plates were prepared. The 100 mg/ml and 200 mg/ml of the Acetone, Benzene, Methanol, Water solvent extracts were prepared by using the respective solvents. Then 200µl of the bacterial suspension was introduced into the sterile plates and spreading the bacteria using L-Rod to get an even culture all over the plates. 6 mm Discs was prepared from Whatman No.1 filter paper and it also autoclaved. A plate comprises of four discs, one is positive control, one is negative control and two for the two different concentrations (100mg/ml, 200 mg/ml) of the same plant extract. The sterile discs are placed above the media and the samples are loaded gently by the following quantity given below. 10 µl of Ampicillin (10 mg/ml) is loaded as positive control in the disc and 10µl of the respective solvents in which the sample is dissolved was used as negative control in the disc. Then 20 µl of the 100 mg/ ml and 200 mg/ ml prepared concentration of the extract is loaded gently in the following discs. Then all the plates are kept for incubation at 37°C for 24 hours. The higher concentration Acetone extract, Benzene extract, Methanol extract and Water extract of the *O. sanctum* shows activity against the tested pathogens by the following order from the highest zone of inhibition *P. aeruginosa* (8.5 mm), *S. typhi* (5 mm) and *E. coli* (3.5 mm) for Acetone extract, *E. coli* (4 mm) only for Benzene extract, whereas *P. aeruginosa* (14 mm), *S. typhi* (8 mm) and *E. coli* (7 mm) for Methanol extract and there is no activity for the water extract was observed for all the three pathogens (Table 2 & Fig. 2). Methanolic extract of *ocimum tenuiflorum* possess antimicrobial potential against both gram positive and gram negative bacteria. The present study provides evidence that solvent extract of *ocimum tenuiflorum* contains medicinally important bioactive compounds as observed by L. Srinivas Naik *et al*., (2015) [17] & Bilal Ahmad Tantry *et al*., (2016) [3] and this justifies the use of plant species as traditional medicine for treatment of various diseases.

3.3 Antioxidant activity

The antioxidant activity of the *O. sanctum* Acetone, Benzene, Methanol and Water solvent extract's tested for antioxidant activity by using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) method and then compared with standard Ascorbic Acid. Antioxidant activity is very important in counteracting the deleterious effect of free radical in food biological system. The DPPH alcohol solution is a deep purple colour with an absorption peak at 517 nm which disappears in the presence of radical scavengers in the in the reactive system. The scavenging capacity of biological reagents on DPPH free radical can be expressed as its antioxidant capability. Evidences gathered in recent year suggest the involvement of free radicals and other oxidants as the major cause of oxidative stress that lead to a variety of diseases and disorders. This led to an increasing interest in natural products having antioxidant properties. Plants have been considered as richer sources of antioxidants. In our study the antioxidant activity of the *O. sanctum* solvents extracts was high to the Methanol followed by Benzene, Acetone, Water extracts (Table 3). The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that

could have great importance as therapeutic agents and oxidative stress related degenerative diseases. Present investigation concluded that there is a good antioxidant potential of *Ocimum sanctum* as reported by Anjali Soni *et al*., (2013) [16], M. Suriyavathana *et al*., (2017) [8], Kang Zhi Xia *et al*., (2018) [6] and Pooja Sudhakar Rindhe *et al*., (2018) [9].

Table 1: Results of phytochemical analysis

S. No.	Phytochemicals	Test Names	Acetone	Benzene	Methanol	Water
1.	Alkaloids	a) Mayer's test	+	+	+	-
		b) Wagner's test	+	+	+	-
2	Amino Acid	Xanthoprotein test	+	+	+	+
3	Carbohydrate	Molish's test	+	+	+	+
4	Phenol	a) $FeCl_3$ test	+	+	+	+
		b) Potassium-di-chromate	+	+	+	+
5	Flavonoids	Alkaline reagent test	+	+	+	-
6	Tannins	$FeCl_3$ test	-	-	-	-
7	Saponin	Foam test	-	+	-	+
8	Terpenoids	a) Salkowskis test	+	+	+	+
		b) Liberman burchard's test	+	+	+	+
9	Phlobatanins	1%Hydrochloric acid test	-	-	-	-
10	Quinones	Hydrochloric acid test	-	-	-	-
11	Coumarin	Sodium hydroxide test	+	+	+	-
12	Glycoside	Kellar-Kilanis test	+	+	+	-
13	Anthocyanins	Sulphuric acid test	-	-	-	-

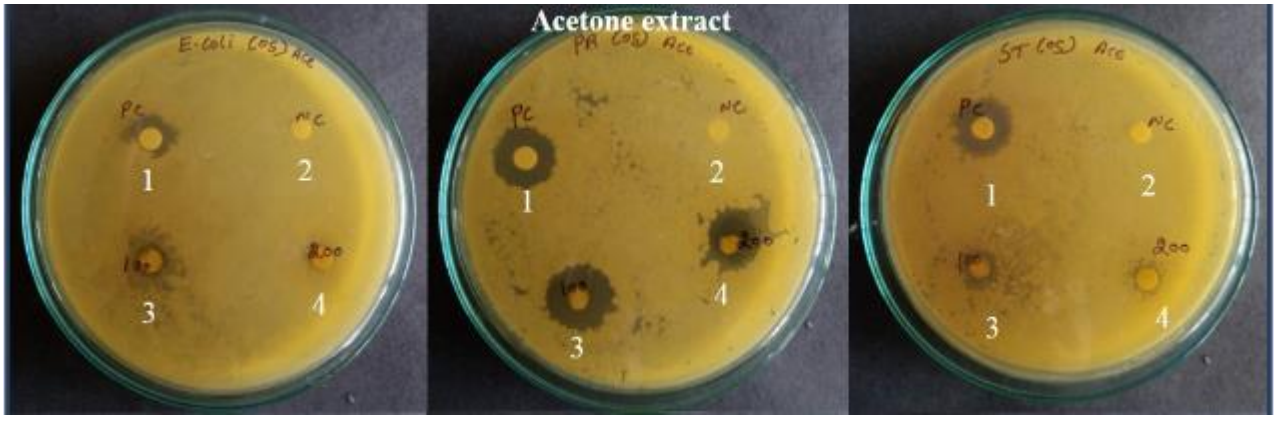
Presence (+) Absence (-)

Table 2: Antibacterial activity of *Ocimum sanctum*

Name of the Microorganism	Solvents	Zone of Inhibition in MM			
		Positive control	Negative control	100 mg/ml	200 mg/ml
Escherichia coli	Acetone	6 mm	-	3 mm	3.5 mm
	Benzene	5 mm	-	-	4 mm
	Methanol	8 mm	-	8 mm	7 mm
	Water	5 mm	-	-	-
Pseudomonas aeruginosa	Acetone	8 mm	-	4 mm	8.5 mm
	Benzene	9 mm	-	-	-
	Methanol	8 mm	-	12 mm	14 mm
	Water	9 mm	-	-	-
Salmonella. Typhi	Acetone	8 mm	-	3 mm	5 mm
	Benzene	8 mm	-	-	-
	Methanol	8 mm	-	4 mm	8 mm
	Water	8 mm	-	-	-

Table 3: Optical Density value of *O. sanctum* extracts at 517 nm.

Concentration	Ascorbic Acid	Acetone	Benzene	Methanol	Water
20 µg/ml	0.0159	0.3977	0.3966	0.3616	0.3679
40 µg/ml	0.0143	0.3568	0.3453	0.2900	0.3571
60 µg/ml	0.0125	0.3071	0.2869	0.2153	0.3539
80 µg/ml	0.0108	0.2648	0.2759	0.1814	0.3522
100 µg/ml	0.0077	0.2332	0.2464	0.1397	0.3450
150 µg/ml	0.0070	0.1666	0.1288	0.1110	0.3368
200 µg/ml	0.0059	0.1426	0.1037	0.0748	0.3292
250 µg/ml	0.0049	0.1090	0.0948	0.0532	0.3145



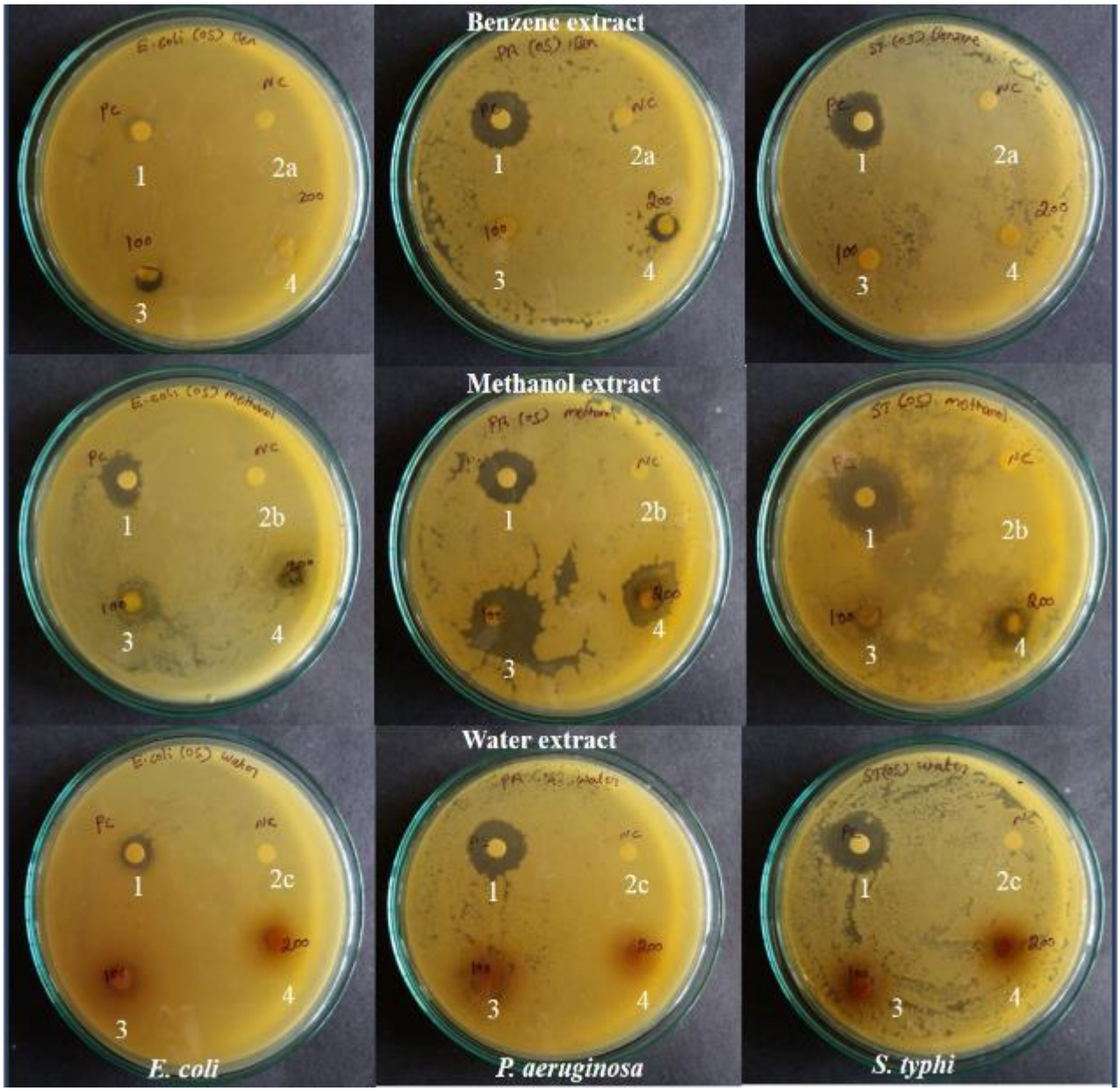


Fig 2: Antibacterial activity of *Ocimum sanctum* leaf extracts. 1 – Positive Control (Ciprofloxacin 1mg/ml), 2- Negative Control (Acetone), 2a - Negative Control (Benzene), 2b - Negative Control (Methanol), 2c - Negative Control (Water), 3 – 100 mg/ml of *Ocimum sanctum* extract & 4- 200 mg/ml of *Ocimum sanctum* extract.

4. Conclusion

The research on *Ocimum sanctum* - Tulasi is globally active, many research findings and explorations are going still in search for the more knowledge about its medicinal values, the basic research has been carried out with the local species in and around from our region. *O. sanctum* was found to be rich in alkaloids and phenolic compounds which shows there will be a high content of medicinal important bioactive compounds. Various extracts shows the present of high concentration of terpenoids compounds which are the main sources of steroids. And the DPPH antioxidant activity of the various extracts shows good results where it shows the presence of rich antioxidants which can serve as a good source for immunity development. A high antibacterial activity was found in methanolic extracts of *O. sanctum* compared to others against bacterial pathogens like *E. coli, Pseudomonas* and *Salmonella* frequent disease-causing microorganisms. With this preliminary research studies, we conclude that the species *O. sanctum* found to be a great source of various medicinally important bioactive compounds as it has good nutritional, antioxidant and antibacterial activities.

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