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Evaluation of phyto constituent and synergistic antibacterial activity of *Ocimum sanctum* extract against some gram-positive and gram-negative species

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ABSTRACT

Plant and plant extracts have been used in traditional medicine since time immemorial. *O.sanctum* has often been cited as one of the main pillars of herbal medicine as it possesses greater medicinal value. It has been proved to be effective against gram positive and gram negative bacteria. This study aimed to determine the *in vitro* antibacterial activity of the medicinal plants *O. sanctum* against the bacterial strains associated with infectious diseases. Extracts of *O. sanctum* were tested for their antibacterial activity against three bacterial species includes *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using the microdilution method. In the present study we found that methanolic extract of leaves of *O. sanctum* was used in combination with ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed fraction inhibitory concentration index (FICI ≤ 0.5). The synergistic antibacterial activity (FICI ≤ 0.5) was observed with combination of methanolic extract of stem and root of *O. sanctum* when used in combination with antibiotic ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed indifferent antibacterial activity (FICI = 1.0 - 4.0). Benzene extract of leaves, stem and root of *O. sanctum* when used in combination with ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed partial synergistic antibacterial activity (FICI = 0.5 - 1.0, 0.5 - 4.0, 1.0 - 4.0) respectively. The synergistic antibacterial activity (FICI ≤ 0.5) was observed with combination of aqueous extract of leaves of *O.sanctum* with ampicillin. While, the aqueous extract of stems and roots of O. sanctum when used in combination with antibiotic ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed partial synergistic antibacterial activity and indifferent antibacterial activity (FICI = 0.5 - 4.0, 1.0 - 4.0) respectively. present investigation indicates clear evidence supporting the traditional use of *O. sanctum* in treating infectious diseases.

KEY WORDS: TULSI, SYNERGISTIC EFFECT, DISEASES, EXTRACTION, FRACTION INHIBITORY CONCENTRATION

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INTRODUCTION

In modern complementary and alternative medical practice, plants are the primary source of therapeutics and each part of the plant, including the seeds, root, stem, leaves, and fruit, potentially contains bioactive components. The search for alternative antimicrobial compounds is an urgent area of biomedical research and extracts derived from plants have long held interest as potential sources of new therapeutic agents. The medicinal use of plants is probably as old as mankind. Plants have continued to be a valuable source of natural products for maintaining human health. Various medicinal plants have been used for years in daily life to treat disease all over the world. One of the remotest works in traditional herbal medicine is Viriksh Ayurveda, compiled even before the beginning of Christian era (Himal *et al*., 2008, Mandave et al., 2014. Jiang et al., 2015 and Sarah et al., 2016).

In the ancient Ayurvedic text, the *Charaka Samhita*, Tulsi has been documented to be of immense use in the treatment of headaches, rhinitis, stomach disorders, inflammation, heart diseases, various forms of poisoning and malaria (Gupta *et al.,* 2002). Each part of the plant has proven to offer protection against various diseases; the aqueous and alcoholic extract from the leaves have various pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antiasthmatic, antiemetic, antidiabetic, hepatoprotective, hypotensive, hypolipidemic, and antistress agents. Further, distillation of the leaves yields oil of the plant which is known to possess antibacterial, antioxidant, and anti-inflammatory properties and is used extensively in the pharmaceutical industry mainly for skin cream preparations (Watson et al., 2012). Different parts of *Ocimum sanctum* Linn (known as Tulsi), a small herb seen throughout India, have been used for various medicinal purposes. The main bioactive components in medicinal plants are considered to be combinations of secondary metabolites (Wu *et al.,* 2016).

Recently Hanaa et al (2016) have been demonstrated that antimicrobial activity of *Ocimum tenuiflorum* essential oil and their major constituents against three species of bacteria. This study was designed at authenticated the traditional use of *Ocimum sanctum* medicinal plants against human pathogenic bacteria, causing a number of human disease including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by assess their *in vitro* antibacterial activity. Due to insufficient screening of the natural compounds and the limited understanding of their mechanism of action against the microorganism the need of the hour is to identify more and more natural compounds which exhibit synergistic behavior with the antibiotics.

MATERIAL AND METHODS

Plant materials *Ocimum sanctum* (Tulsi) were purchased from local market and were authenticated by Dr. S.S. Khan of Botany, Department Saifia Science College Bhopal. The voucher specimen no (J/R201) was deposited at the Herbarium of the Faculty of Botany Department, Saifia Science College Bhopal (M.P.) India. Human disease causing bacteria; *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 96) *Pseudomonas aeruginosa* (MTCC 74) were procured from Institute of Microbial Technology, Chandigarh (IMTECH), India. These bacterial strains were then used for studying the antimicrobial efficiency.

The phytochemical constituents of the *Ocimum sanctum* plant parts (leaf, stem, and root) were extracted in soxhlet apparatus using various solvents Kokate, 1991; Trease and Evans, 1989). Soxhlet extraction is used for separating components based on the difference in the solubility in the solvent. The powdered plant material (50 gm) was placed in the soxhlet extractor flask. 500 ml of the organic solvent was taken in the round bottom flask. The soxhlet extraction was carried out continuously at an appropriate temperature for 6-8 hrs, till colorless extract is collected in the extractor flask. The extract thus obtained was collected in collection bottles and was further subjected to concentration using Rotary vacuum evaporator. After soxhlet extraction the extracts obtained were filtered and then each of the extract was concentrated using rotary vacuum evaporator. The individual extracts were taken in round bottom flask which was heated at appropriate temperature on a water bath. The vapors of the solvent rise in the condenser and after condensation the solvent droplets was collected in the collecting flask.

The resultant sticky mass was collected in the crucible. It was dried at a low temperature in the oven. The solid mass obtained was stored in a suitable volume of 10% dimethyl sulphoxide (DMSO) with a drop of Tween-20. Aqueous extract of the individual plant parts was prepared by decoction method. Filter paper packets of 50 gm of the individual plant parts were prepared. These packets were place separately in 200 ml of hot water contained in bottles. The extraction was carried out for 24 °C with intermittent shaking. The extracts obtained were concentrated and dried. The dried mass obtained was stored in a suitable volume of 10% dimethyl sulphate (DMSO) with a drop of tween-20.

Phytochemical analysis

Chemical test were carried out to identify various constituents using standard method of (Trease and Evans 1989; Harbone, 1973). Mayer reagent was prepared by dissolving 1.36 grams of mercuric chloride in 60 ml of

distilled water and 5 grams of potassium iodide in 20 ml of distilled water. Both the above solution was mixed and volume of the reagent adjusted to 100 ml by distilled water. 1 ml of the plant extract was taken and few drops of mayer reagents were added. Formation of cream colour precipitate was confirms the presence of alkaloid. Fehling solution was prepared by dissolving 4.36 gram of copper sulphate in 50 ml of distilled water and by dissolving 17.3 grams of sodium potassium tartarate and 5 gram of sodium hydroxide in 50 ml of distilled water. Both the solution were mixed prior to use.1 ml of the extract were taken and few drops of fehling solution was added. Formation of red precipitate confirms the presence of carbohydrates and glycosides. Ferric chloride solution was prepared by dissolving 5 grams of ferric chloride in 100 ml of 90% ethanol. 1 ml of extract was taken and few drops of ferric chloride solution were added. Formation of bluish black precipitate confirms the presence of phenolic compounds and tannins.

Ninhydrin solution was prepared by dissolving 0.3 grams of ninhydrin in 100 ml of ethanol. 1 ml of extract was taken and few drops of ninhydrin solution were added and purplish pink colour confirms the presence of proteins and amino acids in extracts. Alkaline reagent was prepared by dissolving 10 grams of sodium hydroxide in 100 ml of distilled water. 1 ml of extract was taken and few drops of sodium hydroxide solution were added. Intense yellow colour confirms the presence of flavonoids. 1 ml of the extract was taken and mixed with few drops of chloroform and few drops of sulphuric acids. A reddish brown colour confirms the presence of terpenoids.1 ml of the extract was taken and diluted with distilled water to 10 ml. Formation of stable foam confirms the presence of saponins.1 ml of the extract was mixed with 5 ml of distilled water mixture was heated and to it was added 5 ml of 1% HCl. Formation of red precipitate confirms the presence of phlobatanins.1 ml of the extract was taken and to it was added in 2 ml of chloroform and 2 ml of concentrated sulphuric acid. Formation of reddish brown layer at the interface confirms the presence of steroids.

Assay of antimicrobial activity using disc diffusion method

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer *et al*., 1996 to assess the presence of antibacterial activities of the various samples. A bacterial suspension was prepared for each of bacteria used for the study. 1 ml of the bacterial suspension was taken in sterile petriplate. To it was added molten nutrient agar media under aseptic conditions and mixed well. It was allowed to solidify for 1 hour to allow the bacteria to grow. These plates were used for sensitivity test. Whatman filter paper disc were impregnated with the samples and were placed on nutrient agar surface. Positive control plate was also prepared with standard antibiotic disc and negative control plate was prepared using DMSO. The plates were then incubated at 37 °C for 24 hrs. After the incubation the plates were examine for zone of inhibition. The inhibition zones were measured using antibiotic zone reader scale

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the plant extracts, antibiotics and combination of plant extracts and antibiotics was determined by diluting the extracts in Nutrient broth to give concentration of 1024, 512, 256, 128, 64, 32, 16, 8, 4 and 2 µg /ml. 2 ml of plant extracts, antibiotics and combination of plant extracts and antibiotics was added to the first tube containing 2 ml of broth. The tube was shaken and 2 ml transferred aseptically to the next tube containing the same quantity of broth. This was done until serial dilution was achieved in the last tube that is the tenth tube. Then 0.1 ml of the MTCC bacterial culture suspension was inoculated into each test tube and they were incubated at 37° C for 24 hours. The absorbance of the tubes was taken in UV-VIS spectrophotometer. The minimum inhibitory concentration was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with the control tube.

Calculate FIC and FIC Index

A widely accepted method, to measure the effect of combination of plant extract and antibiotic is the fractional index. The fractional index is used to identify whether a combination therapy is synergistic, additive or antagonistic. The Inhibitory Concentration is determined using MIC measurements. The fractional Inhibitory Concentration Index (ΣFIC) is the sum of the FICS of each of the plant extract and antibiotics (Rakholiya *et al*., 2015).

Calculations

The FIC was calculated for plant extract and antibiotic as follows:

$$\text{FIC for Plant extract} = \frac{\text{MIC of plant extractin combination}}{\text{MIC of plant extract}}$$

The FIC was calculated for plant extract and antibiotic as follows:

$$\text{FIC for Antibiotic} = \frac{\text{MIC of plant extractin combination}}{\text{MIC of plant extract}}$$

Calculated the summation of FIC (ΣFIC) index for each combination as follows:-

ΣFIC = FIC of Plant Extracts + FIC of Antibiotics

RESULTS

Antibacterial activity of methanolic extracts of *O. sanctum* with ampicillin

The zone of inhibition of the leaf extract against *E. coli*, *P. aeruginosa* and *S. aureus* was 18mm, 15mm and 23mm respectively. The ZOI of the combination of leaf extract of *O. sanctum* and ampicillin were 29mm, 27mm and 30mm. The MIC of the leaf extract was 32µg/ml, 16µg/ml and 32µg/ml against *E. coli*, *P. aeruginosa* and *S. aureus*. The MIC of the combination of leaf extract of *O. sanctum* and ampicillin were 4 µg/ml, 4µg/ml and 4µg/ml respectively. The ZOI of the stem extract against *E. coli*, *P. aeruginosa* and *S. aureus* were 15mm, 10mm and 14mm respectively. The ZOI of the combination of stem extract of *O. sanctum* and ampicillin were 28mm, 21mm and 26mm (Table 1).

The minimum inhibitory concentration values of the stem extract were 16µg/ml, 32µg/ml and 64µg/ml against *E. coli*, *P. aeruginosa* and *S. aureus*. The MIC of the combination of stem extract of *O. sanctum* and ampicillin were 4µg/ml, 16µg/ml and 16µg/ml respectively. The ZOI of the root extract against *E. coli*, *P. aeruginosa* and *S. aureus* were 0mm, 0mm and 10mm respectively. The ZOI of the combination of root extract of *O. sanctum* and ampicillin were 24mm, 22mm and 23mm. The MIC of the root extract was 64µg/ml, 256µg/ml and 128µg/ml against *E. coli*, *P. aeruginosa* and *S. aureus*. The MIC of the combination of root extract of *O. sanctum* and ampicillin were 32µg/ml, 32µg/ml and 16µg/ml respectively (Table 1).

Antibacterial activity of combined effect of benzene extracts of *O. sanctum* with ampicillin

The zone of inhibition of the leaf extract against *E. coli*, P. *aeruginosa* and *S. aureus* was 13mm, 8mm and 15mm respectively. The ZOI of the combination of leaf extract of *O. sanctum* and ampicillin were 26mm, 21mm and 28mm. The MIC of the leaf extract was 32µg/ml, 16µg/ml and 32µg/ml against *E. coli*, P. *aeruginosa* and *S. aureus*. The MIC of the combination of leaf extract of *O. sanctum* and ampicillin were 8µg/ml. The ZOI of the stem extract against *E. coli*, P. *aeruginosa* and *S. aureus* was 11 mm, 0 mm and 10 mm respectively. The ZOI of the combination of stem extract of *O. sanctum* and ampicillin were 25 mm, 20 mm and 22mm (Table 2).The minimum inhibitory concentration values of the stem extract were 64µg/ml, 128µg/ml and 32µg/ml against *E. coli*, P. *aeruginosa* and *S. aureus*. The MIC of the combination of stem extract of *O. sanctum* and ampicillin were 8µg/ml, 16µg/ml and 16µg/ml respectively. The ZOI of the root extract against *E. coli*, P. *aeruginosa* and *S. aureus* was 0mm, 0mm and 8mm respectively.The ZOI of the combination of root extract of *O. sanctum* and ampicillin were 24 mm, 22 mm and 23 mm. The MIC of the root extract was 64 µg/ml, 32µg/ml and 64 µg/ml against *E. coli*, *P. aeruginosa* and *S. aureus*. The MIC of the combination of root extract of *O. sanctum* and ampicillin were 16µg/ml, 16µg/ml and 32µg/ml respectively (Table 2).

Antibacterial activity of combined effect of aqueous extracts of *O. sanctum* with ampicillin

The zone of inhibition of the leaf extract against *E. coli*, P. *aeruginosa* and *S. aureus* was 17mm, 14mm and 20mm respectively. The ZOI of the combination of leaf extract of *O. sanctum* and ampicillin were 28mm, 26mm and 29mm. The MIC of the leaf extract was 16 µg/ml, 64µg/ml and 32µg/ml against *E. coli*, P. *aeruginosa* and *S. aureus*. The MIC of the combination of leaf extract of *O. sanctum* and ampicillin were 2µg/ml, 16µg/ml and 8

Table 1: Zone of Inhibition, Minimum Inhibitory Concentration and Fractional Inhibitory Concentration of methanolic extract of leaf, stem and root of *O. sanctum*.

Plants material	Zone of Inhibition (mm)			Minimum Inhibitory Concentration in µg/ml (MIC)			Fractional Inhibitory Conc. FIC & Σ FIC		
	E. coli	*P. aeruginosa*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. aureus*
Leaf	18	15	23	32	16	32	0.125	0.250	0.125
Ampicillin	23	21	22	16	16	32	0.250	0.250	0.125
Leaf with Ampicillin	29	27	30	4	4	4	0.375	0.500	0.250
Stem	15	10	14	16	32	64	0.250	0.500	0.250
Ampicillin	23	21	22	16	32	32	0.250	0.500	0.500
Stem with Ampicillin	28	21	26	4	16	16	0.500	1.000	0.750
Root	-	-	10	64	256	128	0.500	1.000	1.500
Ampicillin	23	21	22	32	32	16	0.125	1.000	1.125
Root with Ampicillin	24	22	23	32	32	16	0.125	1.000	1.125

Table 2: Zone of Inhibition, Minimum Inhibitory Concentration and Fractional Inhibitory Concentration of Benzene extract of leaf, stem and root of *O. sanctum*.

Plants material	Zone of Inhibition (mm)			Minimum Inhibitory Concentration in µg/ml (MIC)			Fractional Inhibitory Conc. FIC & Σ FIC		
	E. coli	P. aeruginosa	S. aureus	E. coli	P. aeruginosa	S. aureus	E. coli	P. aeruginosa	S. aureus
Leaf	13	8	15	32	16	32	0.250	0.500	0.250
Ampicillin	23	21	22	16	16	32	0.500	0.500	0.250
Leaf with Ampicillin	26	21	28	8	8	8	0.750	0.1000	0.500
Stem	11	-	10	64	128	32	0.125	0.125	0.500
Ampicillin	23	21	22	16	16	32	0.500	0.1000	0.500
Stem with Ampicillin	25	20	22	8	16	16	0.625	1.125	0.1000
Root	-	-	8	64	32	64	0.250	0.500	0.500
Ampicillin	23	21	22	16	16	32	1.000	1.000	1.000
Root with Ampicillin	24	22	23	16	16	32	1.250	1.500	1.500

µg/ml respectively. The ZOI of the stem extract against *E. coli*, P. *aeruginosa* and *S. aureus* was 12 mm, 0 mm and 16 mm respectively. The ZOI of the combination of stem extract of *O. sanctum* and ampicillin were 25mm, 22mm and 28mm (Table 3).

The minimum inhibitory concentration values of the stem extract was 32µg/ml, 32µg/ml and 32µg/ml against *E. coli*, P. *aeruginosa* and *S. aureus*. The MIC of the combination of stem extract of *O. sanctum* and ampicillin were 8µg/ml, 16µg/ml and 8µg/ml respectively. The ZOI of the root extract against *E. coli*, P. *aeruginosa* and *S. aureus* was 9mm, 0mm and 8mm respectively. The ZOI of the combination of root extract of *O. sanctum* and ampicillin were 24mm, 22mm and 23mm. The MIC of the root extract was 32µg/ml, 32µg/ml and 256µg/ml against *E. coli*, P. *aeruginosa* and *S. aureus*. The MIC of the combination of root extract of *O. sanctum* and ampicillin were 16µg/ml, 16µg/ml and 32 µg/ml respectively (Table 3).

Analysis of the Plant Extracts *O. sanctum*

Pytochemical screening of the leaf, stem and root of *Ocimum sanctum* L. were conducted and its results showed presence of different constituents in methanolic, benzene and aqueous extracts. (Table 4). Methanolic extracts showed the presence of alkaloids, carbohydrate, glycosides, phenolic compounds, tannins, proteins, amino acids, flavonoids, terpenoids, saponins and steroids. In

Table 3: Zone of Inhibition, Minimum Inhibitory Concentration and Fractional Inhibitory Concentration of Aqueous extract of leaf, stem and root of *O. sanctum*.

Plants material	Zone of Inhibition (mm)			Minimum Inhibitory Concentration in µg/ml (MIC)			Fractional Inhibitory Conc. FIC & Σ FIC		
	E. coli	P. aeruginosa	S. aureus	E. coli	P. aeruginosa	S. aureus	E. coli	P. aeruginosa	S. aureus
Leaf	17	14	20	16	64	32	0.125	0.250	0.250
Ampicillin	23	21	22	16	32	32	0.125	0.500	0.250
Leaf with Ampicillin	28	26	29	2	16	8	0.250	0.750	0.500
Stem	12	-	16	32	32	32	0.250	0.500	0.250
Ampicillin	23	21	22	16	32	32	0.500	0.500	0.250
Stem with Ampicillin	25	22	28	8	16	8	0.750	0.1000	0.500
Root	9	-	8	32	32	256	0.500	0.500	0.125
Ampicillin	23	21	22	16	32	32	1.000	0.500	1.000
Root with Ampicillin	24	22	23	16	16	32	1.500	1.000	1.125

Table 4: Phytochemical constituents present in methanolic, benzene and aqueous extracts of leaf, stem and root of *O. sanctum.*

Plant Constituents	Leaf			Stem			Root		
	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous
Alkaloids	+	-	+	+	-	-	+	-	+
Carbohydrates Glycosides	+	+	+	+	+	+	+	-	+
Phenolics compounds Tannins	+	-	+	-	-	-	-	-	-
Proteins Amino acids	+	+	+	+	+	+	-	-	+
Flavonoids	+	-	+	-	-	-	-	-	-
Terpenoids	+	-	+	-	-	-	+	-	+
Saponins	+	-	-	+	-	+	+	-	+
Phlobatannins	-	-	-	-	-	-	-	-	-
Steroids	+	-	-	+	-	-	+	-	-

benzene extract carbohydrate, glycosides, phenols, tannins, proteins and amino acids were present. In aqueous extract along with above components terpenoids and saponins were also present. Stem extract of *O. sanctum* in methanol showed presence of alkaloids, phenols, tannins, flavonoids, terpenoids, saponins and phlobatannin while extraction with benzene solvent showed presence of flavonoids only. In aqueous extract phenols, tannins, flavonoids, terpenoids, saponins and phlobatannins were present. Methanolic extracts of root showed the presence of the different phytochemical constituents viz. alkaloids, carbohydrates, glycosides, phenols, tannins, saponins, phlobatannins and steroids. Extraction with benzene showed presence of carbohydrate, glycosides, phenols and tannins. Aqueous extracts of the roots showed the presence of alkaloids, carbohydrates, glycosides, phenols, taninns,saponins and phlobatanins (Table 4).

DISCUSSION

In this study, we attempted to obtain information on the antimicrobial efficacy of *Ocimum sanc*, particularly against pathogens namely *E. coli*, *S. aureus* and *P. aeruginosa,* as these microbes are more commonly associated with initiation and progression of various pathogenic diseases, especially aggressive periodontitis. Synergism was found when methanolic extract of leaves of *Ocimum sanctum* was used in combination with antibiotic ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* bacterial species (FICI ≤ 0.5). The results showed that ocimum leaves extract showed good inhibition against the bacterial strains. This observed antimicrobial activity could be explained by the fact that plant extract may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. The interaction of plant extract with microbial cytoplasmic components and nucleic acids can inhibit the respiratory chain enzymes and interferes with the membrane permeability limiting the development of bacteria. It is also possible that extract not only interact with the surface of membrane but can also penetrate inside the bacteria. The synergistic antibacterial activity (FICI ≤ 0.5) was observed with combination of methanolic extract of stem of *Ocimum sanctum* with ampicillin showed partial synergistic antibacterial activity (FICI = 0.5 - 1.0) . Methanolic extract of root of *Ocimum sanctum* when used in combination with antibiotic ampicillin, against *E. coli* , *S. aureus* and *P. aeruginosa* showed indifferent antibacterial activity (FICI = 1.0 - 4.0).

Our findings are in agreement with Mishra and Mishra, (2011) who studied antibacterial activity of the aqueous, alcoholic, chloroform extract and oil obtained from leaves of *Ocimum sanctum* against *E.coli, P.aeruginosa, S. typhimurium* and *S.aureus*. Extract obtained from *Ocimum sanctum* were observed equally effective against pathogenic gram positive and gram negative bacteria. Benzene extract of leaves of *Ocimum sanctum* when used in combination with antibiotic ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed partial synergistic antibacterial activity (FICI = 0.5-1.0). Benzene extract of stems of *Ocimum sanctum* when used in combination with antibiotic ampicillin, against *E. coli* , *S. aureus* and *P. aeruginosa* showed partial synergistic antibacterial activity and indifferent antibacterial activity (FICI = 0.5 - 4.0). Benzene extract

of roots of *Ocimum sanctum* when used in combination with antibiotic ampicillin against *E. coli* , *S. aureus* and *P. aeruginosa* showed indifferent antibacterial activity (FICI = 1.0 - 4.0) These results are broadly similar to those of studies that used disk diffusion or optical density reduction methods; however, there are differences in reported activity toward Gram positive and Gram negative bacteria, (Helen et al 2011; Poole et al 2011 and Hanaa et al 2016).

The synergistic antibacterial activity (FICI ≤ 0.5) was observed with combination of aqueous extract of leaves of *Ocimum sanctum* with ampicillin. Aqueous extract of stems of *Ocimum sanctum* when used in combination with antibiotic ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed partial synergistic antibacterial activity and indifferent antibacterial activity (FICI = 0.5 - 4.0). Aqueous extract of roots of *Ocimum sanctum* when used in combination with antibiotic ampicillin against *E. coli*, *S. aureus* and *P. aeruginosa* showed indifferent antibacterial activity (FICI = 1.0 - 4.0). This is in agreement with many literatures reporting of differences in the activities of extracts obtained from the same morphological part of a plant using different solvents.

Similar findings have been reported by Ahmad and Aqil, (2007) who found synergistic interaction between crude extracts of Indian medicinal plants and antibiotics against extended spectrum β lactamase producing multidrug-resistant enteric bacteria. Similar observation was found by Sajjanshetty *et al.,* (2016) who reported the antimicrobial efficacy of *Ocimum sanctum* leaf extract on periodontal pathogens it was observed *Ocimum sanctum* i extracts showed antimicrobial activity against *A. actinomycetemcomitans*, similar to doxycycline with similar inhibition zones ($P > 0.05$). *P. gingivalis* and *P. intermedia*, however, exhibited resistance to Tulsi extract that showed significantly smaller inhibition zones ($P < 0.05$).

Methanolic extracts of the leaf showed the presence of alkaloids, carbohydrate, glycosides, phenolic compounds, tannins, proteins, amino acids, flavonoids, terpenoids, saponins and steroids. In benzene extract carbohydrate, glycosides, phenols, tannins, proteins and amino acids were present. In aqueous extract along with above components terpenoids and saponins were also present. Phytochemical constituents such as steroids, alkaloids, flavonoids, tannins, phenol, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores (Bonjar *et al.,* 2004). These secondary metabolites exert antimicrobial activity through different mechanisms. Secondary metabolite alkaloids are one of the largest groups of phytochemicals in plants found in all of extract of *Ocimum sanctum*. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria, tannins have been found to form irreversible complexes with prolinerich protein resulting in the inhibition of cell protein synthesis (Sibanda and Okoh 2007).

Stem extract of *O. sanctum* in methanol showed presence of phenols, tannins, flavonoids, terpenoids, saponins and phlobatannins while extraction with benzene solvent showed presence of flavonoids only. In aqueous extract phenols, tannins, flavonoids, terpenoids, saponins and phlobatannins were present. Methanolic extracts of root showed the presence of the different phytochemical constituents viz. Alkaloids, carbohydrates , glycosides, phenols, tannins, saponins, phlobatannins and steroids. Extraction with benzene showed presence of carbohydrate, glycosides, phenols and tannins. Aqueous extracts of the roots showed the presence of alkaloids, carbohydrates, glycosides, phenols , tannins , saponins and phlobatannins .Eugenol (l-hydroxy-2-methoxy-4-allylbenzene) the active constituent present in *O. sanctum,* is mainly responsible for the therapeutic potential of the plant (Prakash and Gupta, 2005) and the other important constituents include carvacrol, linalool, methyl eugenol , caryophyllene , methyl chavicol and ursolic acid as lead compounds in the composition of *O. sanctum*, (Mohan et al., 2011).

As *Ocimum* is widespread in India, it can be recommended as an easily available and renewal source of antimicrobial agent instead of synthetic chemicals. The present findings indicate that *Ocimum* possesses compounds with antimicrobial properties against pathogenic microorganisms. It is quit safer to use as an herbal medicine as compare to chemically synthesized drug. *Prostanthera* species, like many other australian plants, have been shown to have essential oils with potent antimicrobial activity. Essential oils from the desert species *P. centralis* have been shown to be effective against gram-positive bacteria with MICs against *S. aureus* of approximately 0.1 mg/ml (Collins *et al.,* 2014).

On the other hand, streptomycin sulfate and chloramphenicol used as positive controls showed strong antibacterial activities against both Gram-positive and Gram-negative bacteria like as the results of previous studies (Khan *et al.,* 2014). All the plants parts were extracted with methanol, benzene and aqueous because these considered as the best solvent for the extraction of antimicrobial substances and may contain diverse chemical compounds with biological activity (Robles *et al.,* 2013; Tekwu *et al.,* 2012). The alcoholic extract has greater effect as compared to Benzene and aqueous extract which may be due to the fact that alcohol is comparatively a better solvent as compared with water and benzene for extraction of phytochemical (Levy and Marshall, 2004). Plants antimicrobials have been found

to be synergistic enhancer in that they have little antimicrobial property alone but when they are taken concurrently with standard drug enhances the effect of antibiotics (Chanda and Rakholiya 2011).

CONCLUSION

In ancient and modern era, aerial parts of herbs have been generally used for the cure of crucial health care and variety of ailment across the world depends on geographical cultivation. Leaves of *O. sanctum* play a vital role in health care system due to containing of certain phytochemical. Overall results of current study reflect that highest antimicrobial activities were determined against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Among selected studied medicinal plant material, *O. sanctum* leaf showed more antibacterial activity.

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