Publications

Available Online

J. Sci. Res. **13** (1), 195-208 (2021)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Comparative Antimicrobial Study of Different Parts of *Ocimum americanum* L., *O. basilicum* L. and *O. sanctum* L. in Comparison to Standard Antibiotics Collected from Dibrugarh, Assam

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Received 1 June 2020, accepted in final revised final revised form 9 October 2020

Abstract

Lamiaceae members are commonly used in ethno-medicinal practices of our country. The in-vitro antibacterial and antifungal activity of various solvents extracts of different aerial parts of *Ocimum americanum* L., *O. basilicum* L. and *O. sanctum* L. were assessed on standard bacterial and fungal strains using standard laboratory methods. Extracts from *O. americanum* have inhibitory activity against *B. subtilis*, *B. cereus* and *S. aureus*. Acetone extracts of *O. basilicum* and *O. sanctum* were more potent, exerting significant inhibitory activities against majority of the bacteria investigated. Acetone extract of young inflorescence of *O. americanum* showed highest antibacterial activity against *B. cereus* (14 ± 1 mm) which was also higher than the inhibition of standard Clotrimazole (10 mcg) (10 ± 1 mm). Hot petroleum ether extract of mature leaves of *O. basilicum* showed highest activity against *E. coli* (16 ± 2 mm) which was also higher than the inhibition of Ampicillin, Streptomycitin, Erythromycin. Petroleum ether extract of young leaves of *O. sanctum* recorded highest inhibition against *P. vulgaris* (20 ± 2 mm). In various cases acetone extract of the plants recorded antifungal activity against *C. albicans*. Presence of tannins, flavonoids, saponins, phenols was recorded in all the parts of the plants.

Keywords: *Ocimum spp.*; Antibacterial; Antifungal; Phytochemical.

doi: http://dx.doi.org/10.3329/jsr.v13i1.47322 J. Sci. Res. **13** (1), 195-208 (2021)

1. Introduction

Millions of people are dependent on various antibiotics to live a disease free life. But some multi-drug resistant pathogens are creating some problems to these existing

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antibiotics, creating some undesirable side effects [1-6]. Low resistance power against infectious diseases cause hospitalization, risk of mortality and loss of large amount of money which is not affordable for all of us [3,5,7-9]. Regular use of antibiotics against diseases causes resistance to the causal pathogens. The problem of resistance against these pathogens needs to develop drugs from various other medicinal plants. Phytochemicals present in plants are responsible for some physiological action in plant and in our body also. Many of these secondary metabolites have medicinal value, digestion stimulating, anti-inflammatory, hypolipidemic, antimutagenic and anticarcinogenic properties [10-25]. Among these phytochemicals, some are responsible for their potential antimicrobial activity against various micro-organisms which are more effective and relatively cheaper than modern medicine.

This study was designed to determine the antimicrobial potentiality of different aerial parts of three medicinal plants from Lamiaceae family *Ocimum americanum* L., *O. Basilicum* L. and *O. Sanctum* L., which are commonly known as tulsi, against some standard bacterial and fungal strains. Study and use of different parts will help in sustainable management of these medicinal plants and to save them from extinction. Phytochemical screening was carried out to identify biologically active phytoconstituents. Investigation was carried out on crude extracts. The samples were extracted in different solvents to know the potentially useful extract of the plants. It is hoped that the active parts of the plants having more antimicrobial activity will provide useful information for discovering new compounds with better activity.

O. americanum has antibacterial, insecticidal, antimicrobial, antioxidant, anthelmintic and anti-diabetic properties and used in cold, fever, parasitic infestations, inflammation of joints, headaches, skin diseases, lowering blood glucose, dysentery and diarrhoea, reduce constipation and lipid peroxidation [26-30]. *O. basilicum* is used to strengthen stomach, removal of mucous secretions from the bronchial tubes, protect the alimentary canal and relieves inflammation, used for the treatment of diarrhoea, dysentery, chronic constipation, whooping cough, analgesic, bowels in children, various other intestinal problems, stomach cramps, vomiting, gonorrhoea, in ring worms, scorpion sting, poor digestion, nausea, migraine, depression, insomnia, kidney malfunction, bacterial infection and skin infections [26,31-36]. *O. sanctum* is antioxidant, antibiotic, antiatherogenic, immunomodulatory, anti-inflammatory, analgesic, antiulcer, chemopreventive, hepatoprotective and antipyretic properties [37,38]. The plant is used in various ailments as wound, bronchitis, liver disease, catarrhal fever, lumbago, hiccough, ophthalmia, gastric disorders, genitourinary disorders, skin and heart diseases, eye diseases, tooth disorders, sore throats, mouth infection, malaria, dengue, asthma, influenza, kidney stone, headache, improve memory, rheumatism, pyrexia, psychosomatic stress disorders, in various children's ailments and against insect bite [39-46].

The various species of *Ocimum* from Lamiaceae family are considered as 'holy' plants in our society. They are found in each and every family and are worshiped like 'God'. These commonly found plants of Lamiaceae family have various medicinal properties as described in literature. The various properties of plants differ in different places which

might be due to the habitat and climatic condition of that place. The study was conducted to compare the differences in these properties with other literature which were done in other places.

2. Materials and Methods

2.1. *Sample collection*

Flowering branches of plants (Fig. 1) were collected and brought to the laboratory. Different parts (young and mature leaves, inflorescence and stem) were separated and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at room temperature. The stems were sliced before allowed to dry. After removal of surface water, the materials were wrapped with brown paper and allowed sun drying for complete dryness (less than 1-2 % moisture content). The materials were grounded to fine powder using mortar and pestle and then in electric grinder. The fine powder was kept in air tight bottles for further analysis.

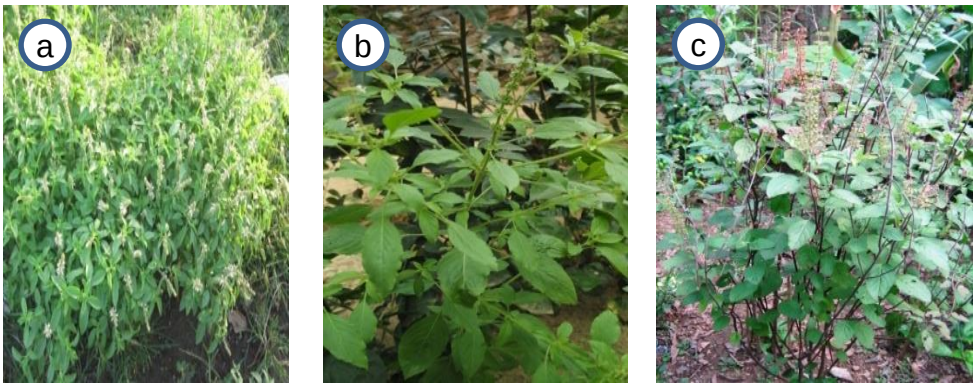

Fig. 1. Digital photographs of three medicinal plants from Lamiaceae family: a) *O. americanum* L., b) *O. basilicum* L. and c) *O. sanctum* L.

2.2. *Preparation of extracts*

Extracts were prepared in five solvents viz., water, methanol, ethanol, acetone and petroleum ether by cold maceration methods and are known as cold extracts. The solvents were selected on the basis of polarity level and their extraction ability. Extracts were collected by soaking 10 g of air dried powder in 500 mL of solvent (except water) for 72 h with intermittent shaking. The extracts were filtered through Whatman no. 1 filter paper into pre-weighed beakers. The filtrate was dried on water bath to obtain a dried mass. The water extract was prepared by soaking 10 g of powder in 500 mL distilled water for 48 h with intermittent shaking. The soaking for 72 h caused fungal growth. The solution was filtered through Whatman no. 1 filter paper. The filtrate was dried to sticky mass using

water bath. The extracts were kept in air tight glass bottles at 5 °C for further analysis. Hot petroleum ether extract was also prepared using soxhlet extractor and antimicrobial activity of the extract was done to observe the difference in activities of both cold and hot petroleum ether extract.

The dried extracts were dissolved in DMSO (dimethyl sulfoxide) to obtain sample solution at 1 mg/mL of concentration. Aqueous extracts were dissolved in distilled water at 1 mg/mL of concentration.

2.3. *Antimicrobial activity assay of the sample extracts*

Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method described by Nair *et al.* [47] using 6 mm borer. The intensity of the activity was determined by measuring the diameter of the zone of inhibition (ZOI) comparing with some standard antibiotics.

Gram positive and gram negative bacterial strains and fungal strains were used in this experiment to know the antimicrobial activity of the sample extracts.

a) Gram positive bacterial strains- *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus Epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744).
b) Gram negative bacterial strains- *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439).
c) Fungal strains- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947).

Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and fungal strains on PDA (Potato Dextrose Agar) slants and stored in freeze. Strains were regularly sub-cultured using nutrient broth for bacterial strains and Potato Dextrose Broth for fungal strains.

The antibacterial activity was assayed by measuring the diameter of the ZOI formed around the well [48]. The resulting ZOI will be uniformly circular as there will be a confluent lawn of growth. The antifungal effect was seen as crescent shaped ZOI [49].

2.4. *Qualitative phytochemical analysis*

Qualitative analysis for detection of tannins, phlobatannins, flavonoids, saponins, alkaloids, cardiac glycosides, terpenoids, steroids, anthraquinone, free anthraquinone, carotenoids and reducing sugar were performed using standard laboratory methods.

2.5. *Statistical analysis*

All the experiments were done in triplicate and mean and standard deviation (SD) was calculated and presented in '±' form.

3. Results and Discussion

The results of antimicrobial activity study of the sample extract of *O. americanum*, *O. basilicum and O. sanctum* are presented in Tables 1 to 3 and standard antibiotics in Table 4. Extracts from the plant recorded good antimicrobial activity against *B. subtilis*, *B. cereus*, *S. aureus* and *P. vulgaris*. Acetone extract of young inflorescence of *O. americanum* showed the highest antibacterial activity against *B. cereus* (14 ± 1 mm) which was also higher (10 ± 1 mm) than the inhibition of standard of Clotrimazole (10 mcg). The sample extracts of *O. basilicum* did not recorded antifungal activity against *C. albicans* and *P. chrysogenum*. Out of twenty extracts from *O. basilicum* only four extracts recorded inhibition against *E. faecalis*. The more number of phytoconstituents present in oil of inflorescence might be the reason of higher activity. Hot petroleum ether extract of mature leaves of *O. basilicum* showed the highest activity against *E. coli* (16 ± 2 mm) which was also higher than the inhibition of Ampicillin (10 mcg) (10 ± 0 mm), Streptomycitin (10 mcg) (12 ± 0 mm), Erythromycin (15 mcg) (12 ± 2 mm). Twenty five extracts of *O. sanctum* did not record inhibition against *P. chrysogenum.* Petroleum ether extract of young leaves of *O. sanctum* recorded the highest inhibition against *P. vulgaris* (20 ± 2 mm) which is higher than the inhibition of standard Ampicillin (10 mcg) (12 ± 2 mm) and Erythromycin (15 mcg) (12 ± 2 mm).

In our study, only acetone extract of young inflorescence of *O. americanum* recorded inhibition against *C. albicans* (10 ± 0 mm). Thaweboon *et al*. [50] showed that essential oils from the plant recorded antimicrobial activity against three different micro-organisms including *C. albicans* using biofilm model. The essential oil from leaves also recorded antibacterial activity against oral bacteria related to periodontal disease [51].

In case of *O. basilicum*, hot petroleum ether extract of mature leaves recorded a significant inhibition against *E. faecalis* and all other extracts recorded no inhibition or negligible inhibition. Prasad *et al*. [52] observed that extracts from *O. basilicum* had no antibacterial activity against *E. faecalis.* In our study, ethanol extract of young leaves and methanol extract of mature leaves recorded inhibition against *S. aureus*. Ethanol, methanol, propanol, chloroform and isoamyl alcohol have activity as 9, 10, 9, 14 and 18 mm respectively against *S. aureus* as studied by Prasad *et al*. [52]. In our study, methanol extract of both young and mature leaves recorded inhibition against *B. subtilis*. Similarly, methanol, propanol, chloroform, petroleum ether and isoamyl alcohol extract recorded activity as 11, 11, 14 and 20 mm respectively against *B. subtilis* as observed by Prasad *et al*. [52]. In our study, various extracts recorded inhibition against *E. coli* and *S. aureus* in agar well diffusion method. Gebrehiwot *et al.* [53] showed that hydrodistilled oil of *O. basilicum* has antimicrobial activity against *E. coli* and *S. aureus* in paper disc diffusion method. But the crude extracts (chloroform:methanol = 1:1) at concentration of 10 and 20 µL, did not recorded activity against the tested microorganisms. Azam and Irshad [54] revealed that ethanol and methanol extract of *O. basilicum* has antibacterial activity against four tested strains. Methanolic, ethanolic and essential oil recorded ZOI as 5, 5 and 4 mm against *S. aureus*; 4, 6 and 8 mm against *E. coli* and 5, 6 and 4 mm against *B.*

subtilis. These results were compared to Ampicillin having ZOI as 6, 5 and 9 against *S. aureus, E. coli* and *B. subtilis* respectively using disc diffusion method. Similarly, in agar well diffusion method also the extracts recorded antibacterial activity against the tested organisms. Methanolic extract recorded ZOI as 7, 4 and 6 mm, ethanolic extract recorded 4, 7 and 4 and essential oils recorded 7, 6 and 7 mm and standard Ampicillin recorded 6, 7 and 9 mm against *S. aureus, E. coli* and *B. subtilis* respectively. They also recorded that there is no significant difference in ZOI in both disc diffusion and agar well diffusion method. In our study, the various extracts of different aerial parts recorded inhibition against *E. coli, S. aureus* and *B. cereus.* Moghaddam *et al*. [55] showed that the essential oil from aerial parts of *O. basilicum* have antimicrobial activity against *E. coli, S. aureus* and *B. cereus.* The Minimum Inhibitory Concentration (MIC) was determined against *E. coli* at concentration 9 µg/mL. In our study, ethanol extract of young leaves recorded inhibition against *E. coli* and *S. aureus*. Shweash *et al*. [36] showed that ethanolic extract of leaves of *O. basilicum* showed antibacterial activity against *E. coli* and ZOI was increased along with increase in concentration. It had lower MIC value (0.312 mg/mL) against *E. coli.* Adam and Omer [56] showed the ethanolic extract of leaves of *O. basilicum* have antibacterial activity against *E. coli, S. aureus* along with other tested bacterial strains. At 100 µg/disc concentration *E. coli* and *S. aureus* showed ZOI as 13.6 and 13.9 mm. Shafique *et al.* [57] also revealed that essential oil from *O. basilicum* have antibacterial activity against both Gram positive and Gram negative bacteria. Raghad *et al*. [58] revealed that ethanolic extract of seeds of *O. basilicum* have antimicrobial activity against *E. coli* (2 mm), *S. aureus* (5 mm), *S. epidermis* (2.5 mm), *P. aeruginosa* (5 mm) and fungi *C. albicans* (3 mm). Shweash *et al*. [59] showed that ethanolic extract of leaves of *O. basilicum* showed antibacterial activity against *E. coli* and ZOI was increased along with increase in concentration. It had lower MIC value (0.312 mg/mL) against *E. coli.* In the present study, antimicrobial activity of cold and hot petroleum ether extract recorded difference in their inhibition against bacterial strains.

In the present study, antimicrobial activity of cold and hot petroleum ether extract recorded difference in their inhibition against bacterial strains. Hot petroleum ether extracts of all the parts (except stem) recorded more inhibition than cold petroleum ether extracts of inflorescence. In our experiment, there is a variation in antimicrobial activities of cold petroleum ether and hot petroleum ether extract of the plants. Sharma *et al.* [60] carried out experiments on antimicrobial activity of ethanol and water extract of leaf and stem of three Lamiaceae members and recorded that ethanol extract have more activity than the water extracts, which may be due to the hot extraction of ethanol using soxhlet apparatus. Zalazare *et al.* [61] recorded that ethanol and hot water extracts of the mushrooms contained higher bioactive substances than cold water extracts. Variable antimicrobial activity was observed in cold and hot water extracts against tested bacterial pathogens [62-65]. According to some other workers, activity of samples also may vary with temperature [66-69]. Traub and Leonhard [70] showed that out of 62 types of antimicrobial material, 25 types were found stable after the heat treatment which is very essential for antimicrobial agents of foods. This kind of differences occurred may be due

to the age of the plant, the time of harvest of the material, method of extraction or may be the thermo-sensitivity of the active compounds.

Table 1. Antimicrobial activity study of the sample extracts of different parts of *Ocimum americanum* L.

Sample	Extracts (mg/mL)	Diameter of ZOI (mm)								
		Bacterial strains							Fungal strains	
		B. subtilis	*B. cereus*	*S. aureus*	*S. epidermis*	*E. coli*	*E. faecalis*	*P. vulgaris*	*C. albicans*	*P. chrysogenum*
Young Leaf	Water	8±1	-	-	-	-	-	-	-	-
	Methanol	8±0	7.8±0.4	8±0	-	-	-	8±1	-	-
	Ethanol	-	8±0	8±0	-	-	-	-	-	-
	Acetone	9.9±1.3	8±1	10.5±2.3	-	-	-	12±2	-	-
	Pet. ether	8±1	8±0	-	-	-	-	8±0	-	-
	Hot Pet. ether	10±2	8±0	8.8±1.2	-	-	-	10±0	-	-
Mature Leaf	Water	-	-	-	-	-	-	-	-	-
	Methanol	8±1	8±0	8±1	8±1	-	-	8±0	-	-
	Ethanol	11±1	-	-	-	-	-	9±1	-	-
	Acetone	-	8±1	-	-	-	-	10±1	-	-
	Pet. ether	-	-	-	-	-	-	8±0	-	-
	Hot Pet. ether	8±0	16.9±1.3	8±1	10±0	-	-	11±1	-	-
Young inflorescence	Water	-	-	-	-	-	-	-	-	-
	Methanol	8±0	-	9±1	10±0	-	-	8±1	-	-
	Ethanol	8±0	8±0	9±1	-	-	-	-	-	-
	Acetone	-	14±1	8±0	-	-	-	-	10±1	-
	Pet. ether	-	8±1	-	-	-	-	-	-	-
	Hot Pet. ether	-	10±1	10±1	-	8±0	8±0	8±0	-	-
Mature inflorescence	Water	-	-	-	-	-	-	-	-	-
	Methanol	8±0	-	-	-	10±2	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-
	Pet. ether	-	-	-	10±0	8±0	-	-	-	-
	Hot Pet. ether	-	8±1	-	-	-	-	-	-	-
Stem	Water	8±0	-	-	-	-	-	-	-	-
	Methanol	10.2±1.4	8±1	11±1	10±1	11±1	8±1	8±0	-	-
	Ethanol	10±1	8±1	8±0	8±1	8±0	9±1	10±1	-	-
	Acetone	10±2	8±1	10±1	8±1	12±0	12±1	12.2±2.1	-	-
	Pet. ether	8±1	8±0	8±0	-	-	-	-	-	
	Hot Pet. ether	-	-	-	-	-	-	-	-	-

Diameter of the cork borer = 6 mm, '-' indicates no inhibition

In our study, acetone extract of young and mature leaves and inflorescence recorded good inhibition against against *B. subtilis* and *S. aureus*. Baskaran [71] carried out antibacterial activity of *O. sanctum* against *E. coli, B. subtilis, S. aureus* and *Klebsiella pneumonia* using various extracts of the plant and recorded good antibacterial activity. The results showed that benzene and chloroform extracts are effective against *S. aureus, K. pneumonia* and *B. subtilis.* There was no activity against *E. coli.* Acetone extract showed strong activity against *K. pneumonia*, but less against *S. aureus* and *B. subtilis.* Chhetri *et al.* [72] showed that 1 % ethanol extract solution of *O. sanctum* recorded ZOI

as 2.2 and 2.1 cm against *E. coli* and *S. aureus* respectively. Singh *et al.* [73] revealed that the aqueous and methanol extracts of *O. sanctum* did not recorded activity against *E. coli*. The extracts showed the largest ZOI (20 and 60 mm respectively) at 200 mg/L against *S. aureus* followed by 100 mg/L and 50 mg/L concentrations (16 mm and 14 mm) respectively of methanol extract. In case of aqueous extract the ZOIs were 11 and 14 mm at 100 and 50 mg/L concentrations. Prasad *et al*. [52] showed that extracts from *O. sanctum* tested did not show any activity against *E. faecalis*. Isoamyl alcohol showed activity of 18 mm against *S. aureus*. Similarly ethanol, methanol, propanol, chloroform and isoamyl alcohol have activity as 10, 10, 11, 13 and 24 mm, respectively against *B. subtilis.* Methanolic leaf extract showed activity against *B. subtilis, S. aureus* and *E. coli* [74]. The antimicrobial activity recorded by the solvent extracts is may be due to the phytochemicals present in the plant.

Table 2. Antimicrobial activity study of the sample extracts of different parts of *Ocimum basilicum* L.

Sample	Extracts (mg/mL)	Diameter of ZOI (mm)								
		Bacterial strains							Fungal strains	
		B. subtilis	*B. cereus*	*S. aureus*	*S. epidermis*	*E.coli*	*E. faecalis*	*P. vulgaris*	*C. albicans*	*P. chrysogenum*
Young Leaf	Water	-	-	-	-	-	-	-	-	-
	Methanol	10±0	8±0	-	-	8±0	-	10±0	-	-
	Ethanol	-	10±1	8±0	8±1	10±1	-	10±0	-	-
	Acetone	10±0	10±1	8±1	8±1	8±0	-	10±0	-	-
	Pet. ether	8±1	8±1	8±1	8±0	-	-	8±0	-	-
	Hot Pet. ether	12±1	8±1	-	-	-	-	10±0	-	-
Mature Leaf	Water	-	-	-	-	-	-	-	-	-
	Methanol	8±1	9±1	8±1	-	-	-	-	-	-
	Ethanol	-	8±0	-	-	-	-	8±1	-	-
	Acetone	-	8±1	-	-	8±0	-	-	-	-
	Pet. ether	8±1	8±1	8±0	-	-	-	10±1	-	-
	Hot Pet. ether	8±0	-	8±0	-	16±2	10±0	8±0	-	-
Inflorescence	Water	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	11±1	-	-
	Ethanol	-	-	-	-	-	-	9±1	-	-
	Acetone	10±0	12±0	10±1	10±0	9±1	8±0	8±0	-	-
	Pet. ether	-	8±1	-	-	12±2	8±0	8±0	-	-
	Hot Pet. ether	10±2	10±1	8±1	10±0	12±0	-	-	-	-
Stem	Water	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	8±0	-	-
	Ethanol	8±0	-	-	-	8±0	-	-	-	-
	Acetone	8±0	10±1	8±1	8±1	8±0	8±0	8±0	-	-
	Pet. ether	-	8±1	8±0	-	-	-	-	-	-
	Hot Pet. ether	-	-	-	-	-	-	-	-	-

Diameter of the cork borer = 6 mm, '-' indicates no inhibition

Table 3. Antimicrobial activity study of the sample extracts of different parts of *Ocimum sanctum* L.

Sample	Extracts (mg/mL)	Diameter of ZOI (mm)								
		Bacterial strains							Fungal strains	
		B. subtilis	*B. cereus*	*S. aureus*	*S. epidermis*	*E. coli*	*E. faecalis*	*P. vulgaris*	*C. albicans*	*P. chrysogenum*
Young Leaf	Water	-	-	-	-	-	-	-	-	-
	Methanol	10±1	-	-	-	-	-	-	-	-
	Ethanol	-	-	8±1	8±1	-	-	11.3±1.1	-	-
	Acetone	-	14.2±2.2	12±0	10.4±2.1	8±1	8±1	12±1	10±1	-
	Pet. ether	-	10±1	10±0	-	-	-	20±2	-	-
	Hot Pet. ether	8±1	10±0	8±1	-	-	-	12±1	-	-
Mature Leaf	Water	-	-	-	-	-	-	-	-	-
	Methanol	-	10±1	8±1	8±1	-	-	-	-	-
	Ethanol	8±0	8±1	8±2	8±1	-	-	8±0	-	-
	Acetone	8±2	14.2±1.4	10±2	10±1	8±0	-	8±0	10.2±1.1	-
	Petroleum ether	-	8±0	8±0	8±1	-	-	-	-	-
	Hot Pet ether	-	-	-	8±0	-	-	8±1	-	-
Inflorescence	Water	-	-	-	-	-	-	-	-	-
	Methanol	8±1	7.8±0.1	-	-	-	-	-	-	-
	Ethanol	-	10±1	-	-	8±0	10±1	-	-	-
	Acetone	10±1	15.3±2.1	10±1	-	10±1	-	-	16±3	-
	Pet. ether	-	10±1	-	8±0	8±1	-	-	-	-
	Hot Pet. ether	14±1	8±0	-	-	-	-	8±1	-	-
Stem	Water	-	-	-	-	-	-	-	-	-
	Methanol	-	8±1	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-
	Acetone	-	-	-	-	8±1	-	8±1	-	-
	Pet. ether	-	-	-	-	-	-	-	-	-
	Hot Pet. ether	-	-	-	-	-	-	-	-	-

Diameter of the cork borer = 6 mm, '-' indicates no inhibition

Table 4. Zone of Inhibition (ZOI) of standard antibiotics for antibacterial and antifungal inhibition.

Standard ↓	Diameter of ZOI (mm)								
	B.subtilis	*B.cereus*	*S.aureus*	*S.epidermis*	*P.vulgaris*	*E.faecalis*	*E.coli*	*C. albicans*	*P. crysogenum*
Chloramphenicol(C) 30 mcg	15±2	-	-	30±0	-	8±0	-		
Tobramycin(TOB) 10 mcg	44±2	24±0	32±0	-	40±4	42±2	35±5		
Clotrimazole (CC) 10 mcg	20±0	10±1	14±0	20±0	-	-	26±0		
Ampicillin(AP) 10 mcg	-	-	-	-	12±2	10±1	10±0		
Streptomycitin (ST) 10 mcg	18±0	-	10±1	-	-	10±0	12±0		
Imipenem(IPM) 10 mcg	66±0	-	-	-	32±2	-	30±1		
Ciprofloxacin(CI) 30 mcg	44±0	32±2	40±4	-	40±4	36±3	22±1		
Streptomycin(S) 25 mcg	-	32±0	28±2	-	22±2	60±2	28±2		

Gentamycin(GEN) 30 mcg	40±0	32±3	30±4	-	-	-	24±2		
Erythromycin(E) 15 mcg	32±2	30±1	28±0	30±0	12±2	48±6	12±2		
Co-trimaxazole(COT) 25 mcg	46±1	-	-	-	30±4	-	24±0		
Nystatin (NS) 50 mcg								-	24±2
Clotrimazole(CC) 10 mcg								11±2	32±0
Ampicillin(AP) 10 mcg								-	46±0

'-' indicates no inhibition

Phytochemical analysis of the plants is presented in Tables 5 to 7. Tannins, flavonoids, saponins, phenols are recorded in all the parts of all the plant. Steroids, glycosides, carotenoids, alkaloids are present in leaves but absent in inflorescence and stem of *O. americanum* and *O. basilicum*. In *O. sanctum*, presence of phytochemicals is recorded in all the parts of the plant. Other workers from various parts of India recorded the presence of alkaloids, saponins, tannins, steroids, flavonoids, reducing sugar, carbohydrate, amino acid, glycosides, protein, phenolic compounds in *O. americanum* [75,76]. Daniel *et al*. [77]; Choudhury *et al.* [75]; Prasad *et al*. [52], Adtani *et al.* [78], Gebrehiwot *et al.* [53], Azam and Irshad [54], Warsi and Sholichah [79], from various places (Tamil Nadu, Odisha, Ethiopia) carried out phyochemical screening of *O. basilicum* and recorded various phytochemicals. Some other workers recorded various phytochemicals in the *O. sanctum* from various places, Tamil Nadu, Odisha, Bhopal, Jaipur, Akola district (MS) [50,60,71,73-75,80-84].

Table 5. Qualitative phytochemical analysis of different parts of *Ocimum americanum* L.

Sample	Tannins	Phlobatannins	Flavonoids	Terpenoids	Steroids	Glycosides	Cardiac Glycosides	Saponins	Anthraquinones	Free Anthraquinones	Carotenoids	Alkaloids	Reducing Sugar	Phenols
Young Leaf	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Mature Leaf	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Young Inflorescence	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Mature Inflorescence	+	-	+	+	+	-	-	+	-	-	-	-	-	+
Stem	+	-	+	-	-	-	-	+	-	-	-	-	-	+

'+' indicates presence, '-' indicates absence

Table 6. Qualitative phytochemical analysis of different parts of *Ocimum basilicum* L.

Sample	Tannins	Phlobatannins	Flavonoids	Terpenoids	Steroids	Glycosides	Cardiac Glycosides	Saponins	Anthraquinones	Free Anthraquinones	Carotenoids	Alkaloids	Reducing Sugar	Phenols
Young Leaf	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Mature Leaf	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Inflorescence	+	-	+	+	-	+	+	+	-	-	-	-	+	+
Stem	+	-	+	+	-	+	+	+	-	-	-	-	+	+

'+' indicates presence, '-' indicates absence

Table 7. Qualitative phytochemical analysis of different parts of *Ocimum sanctum* L.

Sample	Tannins	Phlobatannins	Flavonoids	Terpenoids	Steroids	Glycosides	Cardiac Glycosides	Saponins	Anthraquinones	Free Anthraquinones	Carotenoids	Alkaloids	Reducing Sugar	Phenols
Young Leaf	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Mature Leaf	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Inflorescence	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Stem	+	-	+	+	+	+	+	+	-	-	+	-	+	+

'+' indicates presence, '-' indicates absence

From the above study, it can be concluded that the different parts of these three plants from Lamiaceae family have anti-microbial properties against tested bacterial and fungal strains. Instead of using whole plant, different parts can be used in medicinal practices which will help in sustainable management of these medicinal plants. This will also save the plants extinction.

4. Conclusion

Different extracts of different parts of the plants recorded antimicrobial inhibition against bacteria and fungi. Extracts from the leaves recorded more inhibition than the extracts from inflorescence and stem. Methanol, ethanol and acetone extracts recorded good inhibition than water and petroleum ether extract. The study can be concluded that instead of using the whole plant, different parts can be used in medicinal practices. This will also helps in sustainable management of these medicinal plants.

Acknowledgment

First author acknowledges DST, Govt. of India, for the financial help in the form of INSPIRE Fellowship during the research tenure.

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