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***ORIGINAL RESEARCH ARTICLE***

# *In Vitro* Antibacterial Activity and Phytochemical Analysis of Some Selected Medicinal Plants

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## ABSTRACT

Medicinal plants are extensively used in traditional medicine to cure various infectious diseases in human. The present study was undertaken to investigate *in vitro* antibacterial activity of successively extracted hexane, dichloromethane (DCM), ethyl acetate, ethanol, methanol and aqueous extracts of bulb of *Allium sativum*, leaf of *Eucalyptus citriodora* and *Ocimum sanctum* against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at different test concentration by agar well diffusion method. The test samples were also subjected to detect the presence of phytochemicals. The results of antibacterial activity was analyzed by using one-way analysis of variance (ANOVA) and followed by Least Significant Difference (LSD) test. The study revealed that the extracts possessed antibacterial activity in a dose dependent manner. Among the test plants *E.citriodora* showed better activity against test bacteria. Aqueous extract of *E.citriodora* exhibited significantly ($P < 0.05$) higher effect on *B.subtilis* at the concentration of 0.5 mg / 100 µl. Methanol and aqueous extracts of *E.citriodora* on *B.subtilis*, ethyl acetate and ethanol extract of *E.citriodora* on *S.aureus* and methanol extract of *E.citriodora* on *P. aeruginosa* showed significantly ($P < 0.05$) higher effect at the concentration of 1.0 mg / 100 µl compared to other test extracts. Among the different test samples of *O.sanctum*, ethanol extract produced better inhibition on *B.subtilis*. Ethyl acetate extract of *A.sativum* and aqueous extract of *O.sanctum* showed inhibitory effect on all test bacteria at the concentration ranged from 1.0 mg / 100 µl to 30.0 mg / 100 µl. Phytochemical study revealed that tannins, alkaloids, cardiac glycosides, saponins and terpenoids are present in ethyl acetate and ethanol extracts of *E.citriodora*. All other test samples except hexane extracts contain at least one of the phytochemical tested. This study suggests that ethyl acetate, ethanol, methanol and aqueous extracts of *E.citriodora* ethyl acetate extract of *A.sativum* and ethyl acetate and aqueous extracts of *O.sanctum* can be used for further isolation and purification of active principles.

**Key words:** Medicinal plants, Sequential extraction, Phytochemicals, Antibacterial activity

## INTRODUCTION

Since prehistoric period medicinal plants used in traditional medicine play significant role to heal human diseases and disorders [1]. The medicinal properties of the plants could be credited to the presence of one or more of the active constituents of the plant [2]. It has been reported that the antimicrobial activities of medicinal plants can be due to the presence of phytochemicals such as alkaloids, flavonoids and terpenoids [3]. In recent years in order to discover novel antimicrobial drugs, screening of plants have been accelerated [4]. Therefore the preliminary screening of medicinal plants for antimicrobial activity and for the presence of phytochmicals can establish a flat form for further development on the research of this area.

*Eucalyptus citriodora* (Lemon scented gum) is a tree and belongs to the family Myrtaceae [5]. In traditional medicine essential oil of *Eucalyptus* species has been applied for the treatment of respiratory tract disorders, cold, chest pain, coughs and infections [6, 7]. It was also reported that *E.citriodora* was found to possess antibacterial [8], antifungal [7] and anticancer [5] activities. *Ocimum sanctum* (Holy Basil) is a shrub, belongs to family Labiatae. Different parts of *O. sanctum* are commonly employed for the treatment of fever, bronchitis, cough, arthritis, digestive complaints, and also this plant has anticancer, antidiabetic and antimicrobial properties [9]. *Allium sativum* (Garlic) is a perennial, erect, bulbous plant and belongs to

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family Liliaceae. The juice of bulb is used to treat skin diseases, ear ache and cough [10]. Many studies have been carried out to determine the antimicrobial activities of *A.sativum, O.sanctum* and *E.citriodora*. However, extraction of these plants for antibacterial screening was earlier done using single solvent but in the present study an attempt has been made to investigate *in vitro* antibacterial activity of sequentially extracted solvent extracts of *Ocimum sanctum, Eucalyptus citriodora* and `*Allium sativum* by agar well diffusion method and to correlate the presence of phytochemicals in each extract.

## MATTERIALS AND METHODS

### Collection and identification of plant materials

*Eucalyptus citriodora, Ocimum sanctum* and *Allium sativum* were identified at the Department of Botany, University of Jaffna, Sri Lanka. The fresh and healthy leaves of *E.citriodora* and *O. sanctum* and bulb of *A.sativum* were collected, washed thoroughly under running tap water and dried under sun light. The bulb of *Allium sativum* was cut in to small pieces and allowed for drying. Dried material of each plant was ground into fine powder in an electric blender and was kept in airtight bottles at room temperature until used.

Sequential extraction method was employed to extract the plant powders using different polar solvents from non polar to polar namely hexane, dichloromethane (DCM), ethyl acetate, ethanol, methanol and water [11]. 100 g of dried powder of each plant material was soaked into 300 ml of hexane in an air tight bottle separately and kept on an electric shaker at room temperature for 72 hours. Then these were first filtered through double layered muslin cloth and then using Whatman No.1 filter paper and each filtrate was collected into conical flask separately. Above extraction procedure was repeated thrice with fresh hexane and the extract was concentrated under reduced pressure and low temperature using rotary evaporator. The dried extracts were stored in refrigerator until used for the assay. The residue was dried and further used for dichloromethane extraction followed by ethyl acetate, ethanol, methanol and water similar to the procedure carried out for hexane. The yield percentage of each extract was calculated as follows.

$$\text{Percentage of yield} = \frac{\text{Final weight of dried extract}}{\text{Initial weight of powder}} \times 100$$

### Phytochemical analysis

Each test samples of all three plants was subjected to phytochemical analysis, to detect the presence of saponins, cardiac glycosides, flavonoids [12], tannins, alkaloids [13] and terpenoids [14].

### Test pathogens

Gram negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* and Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* were kindly provided by Department of Microbiology, Faculty of Medicine, University of Jaffna, Sri Lanka. All the test bacteria were stored on nutrient agar slope at 4 ºC. These bacteria were sub cultured for 24 hours before use.

### Test sample preparation

The test concentration of 0.5, 1, 10, 20, 30, 40, 50 and 60 mg / 100 µl were prepared using the solvent mixture of dimethyl sulfoxide (DMSO) and acetone (1:1 v / v).

### Antibacterial assay

*In vitro* antibacterial activity of different solvent extracts obtained by sequential extraction method was studied using agar well diffusion method [15]. Briefly 15 ml of autoclved nutrient agar was cooled down to 40 ºC and mixed with 1 ml of bacterial suspension (1 x $10^6$ cells / ml). The mixed medium was poured into a sterile Petri dish and allowed to set. Wells were made on it using sterile cork borer of 8 mm diameter and each well was filled with 100 µl of each extract.100 µl of Streptomycin (50 µg / 100 µl) and mixture of acetone and DMSO (1:1 v / v) were used as standard and control respectively. The plates were incubated at 37 ºC for 24 hours and antibacterial activity was recorded by measuring the zone of inhibition around the well. Experiments were carried out under biological safety cabinet. Each experiment was repeated thrice and the mean value of inhibition zone was taken.

### Statistical analysis

Diameter of zone of inhibition (excluding well diameter) resulted from replicates were expressed as mean ± standard deviation (SD). The data were analysed by one-way analysis of variance (ANOVA), P value < 0.05 was considered as significant and mean values were compared by using Least Significant Difference

(LSD) test using computer software, SAS system for windows (version 8).

## RESULTS

**Yield percentage:** The data reveled that among the solvent used, ethanol, methanol and aqueous resulted more yield compared to other solvent and the yield was very less in hexane for all three plants (**Table 1**).

**Table 1.Yield percentage of different solvent extracts of test plants obtained by sequential extraction method.**

| Test Plants | Yield Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Hexane | DCM | E.A | Ethanol | Methanol | Aqueous |
| *E citriodora* | 0.824 | 2.960 | 2.280 | 4.212 | 2.998 | 3.812 |
| *O. sanctum* | 0.839 | 1.117 | 1.332 | 3.721 | 4.172 | 3.362 |
| *A. sativum* | 0.451 | 1.920 | 2.800 | 5.200 | 7.210 | 9.540 |

*DCM- Dichloromethane, E.A. Ethyl acetate*

### Antibacterial activity

The results revealed that the inhibitory effect of test samples was dose-dependent as the concentration increased the zone of inhibition was also increased. However some extracts failed to inhibit the growth of test bacteria at the test concentration.Among the test bacteria *B.subtilis* was more sensitive to most of the test extracts, but *E.coli* was less sensitive (**Table 2-5**).

**Table 2: Antibacterial activity of different solvent extracts of test plants against B.subtilis at various concentration.**

| Test plants | Test extract | Concentration (mg / 100 ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 10 | 20 | 30 | 40 | 50 | 60 |
| *Eucalyptus citriodora* | Hexane | - | - | - | - | - | 5.2±0.7$^{h}$ | 10.5±0.2$^{g}$ | 11.3±0.6$^{h}$ |
| | DCM | - | - | - | - | 2.4±0.2$^{i}$ | 5.3±0.2$^{h}$ | 7.3±0.3$^{i}$ | 8.4±0.5$^{k}$ |
| | EA | 2.2±0.2$^{c}$ | 3.3±0.3$^{d}$ | 5.6±0.2$^{e}$ | 5.9±0.1$^{e}$ | 6.3±0.3$^{g}$ | 7.8±0.7$^{g}$ | 8.8±0.4$^{h}$ | 9.6±0.5$^{j}$ |
| | Ethanol | 2.3±0.3$^{c}$ | 5.2±0.4$^{b}$ | 12.4±0.2$^{b}$ | 13.6±0.1$^{a}$ | 14.1±0.2$^{b}$ | 16.2±0.1$^{c}$ | 16.7±0.2$^{d}$ | 18.5±0.3$^{d}$ |
| | Methanol | 3.2±0.1$^{b}$ | 7.3±0.3$^{a}$ | 8.6±0.4$^{d}$ | 11.3±0.4$^{c}$ | 12.2±0.2$^{c}$ | 18.4±0.4$^{a}$ | 21.5±0.3$^{a}$ | 22.9±0.7$^{a}$ |
| | Aqueous | 3.4±0.3$^{a}$ | 7.5±0.1$^{a}$ | 13.1±0.2$^{a}$ | 13.7±0.2$^{a}$ | 14.6±0.1$^{a}$ | 15.3±0.2$^{d}$ | 16.4±0.5$^{d}$ | 17.4±0.4$^{e}$ |
| *Ocimum sanctum* | Hexane | - | - | - | - | - | 5.5±0.3$^{h}$ | 6.5±0.3$^{j}$ | 9.3±0.6$^{j}$ |
| | DCM | - | - | - | - | - | 1.7±0.2$^{j}$ | 2.6±0.1$^{l}$ | 4.3±0.4$^{m}$ |
| | EA | - | - | - | 5.5±0.1$^{f}$ | 10.3±0.2$^{e}$ | 10.9±0.2$^{f}$ | 11.5±0.3$^{f}$ | 14.1±0.1$^{g}$ |
| | Ethanol | 1.5±0.4$^{d}$ | 4.2±0.2$^{c}$ | 8.2±0.3$^{d}$ | 9.5±0.1$^{d}$ | 11.5±0.2$^{d}$ | 16.3±0.2$^{bc}$ | 18.7±0.1$^{c}$ | 19.4±0.3$^{c}$ |
| | Methanol | - | - | - | - | 8.6±0.4$^{f}$ | 12.6±0.4$^{e}$ | 14.7±0.2$^{e}$ | 15.6±0.6$^{f}$ |
| | Aqueous | - | - | - | 4.3±0.2$^{g}$ | 6.2±0.1$^{g}$ | 7.8±0.4$^{g}$ | 8.8±0.1$^{h}$ | 10.3±0.4$^{i}$ |
| *Allium sativum* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | 1.2±0.2$^{j}$ | 3.3±0.5$^{i}$ | 5.5±0.3$^{k}$ | 6.9±0.1$^{l}$ |
| | EA | - | 2.6±0.6$^{e}$ | 11.4±0.4$^{c}$ | 12.2±0.3$^{b}$ | 15.0 ±0.2$^{a}$ | 16.7±0.7$^{b}$ | 19.4±0.9$^{b}$ | 21.3±0.7$^{b}$ |
| | Ethanol | - | - | - | 1.2±0.3$^{h}$ | 3.5±0.6$^{h}$ | 5.5±0.2$^{h}$ | 7.3±0.2$^{i}$ | 8.7±0.5$^{k}$ |
| | Methanol | - | | - | - | - | - | 1.8±0.3$^{m}$ | 3.5±0.2$^{n}$ |
| | Aqueous | - | - | - | - | - | - | - | - |
| **Streptomycin (50 µg / 100 µl)** | | | **24.0 ± 0.1** | | | | | | |
| **Acetone : DMSO (1:1 v / v)** | | | - | | | | | | |

*Values are diameter of zone of inhibition in mm (Mean ± SD), Values with different superscripts in the same column differ significantly (P < 0.05). (-) No Activity, DCM- Dichloromethane, EA – Ethyl acetate,*

*B.subtilis* was inhibited by ethyl acetate, ethanol, methanol and aqueous extracts of *E.citriodora* and ethanol extract of *O.sanctum* even at 0.5 mg / 100 µl (Table 2) and the results produced by the aqueous extract of *E.citriodora* was found to be significantly (P < 0.05) higher. All other test samples except hexane and aqueous extract of *A.sativum* also showed inhibition on *B.subtilis* and the required minimum inhibitive concentration ranged from 1 mg / 100 µl to 50 mg / 100µl. Hexane and aqueous extracts of *A.sativum* failed inhibit the growth of *B.subtilis* even at the highest test concentration (**Table 2**). At 0.5 mg / 100 µl only the DCM extract of *E.citriodora* had ability to inhibit the growth of *S.aureus* but other solvent extracts did not inhibit the growth of *S.aureus* at this concentration. Though *S.aureus* was also inhibited by ethyl acetate, ethanol, methanol and aqueous extract of *E.citriodora* at the concentration of 1 mg /100 µl, ethyl acetate and ethanol extracts had significantly (P < 0.05) higher effect. *S.aureus* was also sensitive to other

solvent extracts of test plants except DCM and ethyl acetate extracts of *O.sanctum* and hexane extract of *A.sativum* at various concentrations ranging from 10 - 50 mg / 100 µl (**Table 3**)

**Table 3: Antibacterial activity of different solvent extracts of test plants against *S.aureus* at various concentration.**

| Test plants | Test extract | Concentration (mg / 100 µl) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 10 | 20 | 30 | 40 | 50 | 60 |
| *Eucalyptus citriodora* | Hexane | - | - | - | - | - | 6.6±0.7$^{g}$ | 12.7±0.2$^{d}$ | 13.4±0.5$^{d}$ |
| | DCM | 2.8±0.3 | 4.5±0.4$^{b}$ | 6.3±0.1$^{c}$ | 6.9±0.2$^{c}$ | 8.2±0.4$^{f}$ | 10.4±0.3$^{d}$ | 10.8±0.3$^{f}$ | 11.4±0.3$^{f}$ |
| | EA | - | 5.5±0.1$^{a}$ | 6.5±0.3$^{c}$ | 6.7±0.1$^{d}$ | 7.3±0.3$^{g}$ | 9.5±0.1$^{e}$ | 12.7±0.3$^{d}$ | 13.8±0.6$^{d}$ |
| | Ethanol | - | 5.3±0.3$^{a}$ | 11.6±0.2$^{a}$ | 14.6±0.3$^{a}$ | 14.7±0.1$^{b}$ | 15.4±0.2$^{b}$ | 10.8±0.1$^{f}$ | 11.4±0.4$^{f}$ |
| | Methanol | - | 4.1±0.1$^{c}$ | 8.3±0.3$^{b}$ | 10.4±0.1$^{b}$ | 13.7±0.1$^{c}$ | 14.6±0.2$^{c}$ | 15.8±0.4$^{b}$ | 18.7±0.3$^{b}$ |
| | Aqueous | - | 2.2±0.1$^{d}$ | 11.3±0.2$^{a}$ | 14.3±0.3$^{a}$ | 15.8±0.1$^{a}$ | 16.2±0.2$^{a}$ | 17.3±0.1$^{a}$ | 20.5±0.3$^{a}$ |
| *Ocimum sanctum* | Hexane | - | - | - | - | - | 3.6±0.3$^{i}$ | 4.4±0.2$^{i}$ | 5.5±0.4$^{j}$ |
| | DCM | - | - | - | - | - | - | - | - |
| | EA | - | - | - | - | - | - | - | - |
| | Ethanol | - | - | - | - | 5.1±0.4$^{h}$ | 5.6±0.2$^{g}$ | 5.9±0.2$^{h}$ | 7.5±0.2$^{j}$ |
| | Methanol | - | - | - | - | 9.1±0.6$^{e}$ | 10.4±0.7$^{d}$ | 14.6±0.2$^{c}$ | 15.7±0.6$^{c}$ |
| | Aqueous | - | - | - | - | 3.6±0.3$^{i}$ | 4.6±0.2$^{h}$ | 6.4±0.2$^{h}$ | 10.4±0.1$^{g}$ |
| *Allium sativum* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | 5.5±0.2$^{h}$ | 6.2±0.2$^{f}$ | 7.9±0.5$^{g}$ | 9.5±0.1$^{h}$ |
| | EA | - | - | 2.4±0.4$^{d}$ | 7.1±0.3$^{c}$ | 9.8±0.4$^{d}$ | 10.8±0.7$^{d}$ | 11.9±0.3$^{e}$ | 12.6±0.3$^{e}$ |
| | Ethanol | - | - | - | - | - | 3.2±0.3$^{i}$ | 4.6±0.1$^{i}$ | 7.6±0.4$^{i}$ |
| | Methanol | - | - | - | - | - | 2.3±0.1$^{j}$ | 4.1±0.1$^{i}$ | 10.0±0.6$^{g}$ |
| | Aqueous | - | - | - | - | - | - | 3.3±0.1$^{j}$ | 4.7±0.2$^{k}$ |
| **Streptomycin (50 µg / 100 µl)** | | **12.7 ± 0.3** | | | | | | | |
| **Acetone : DMSO(1:1 v / v)** | | - | | | | | | | |

*Values are diameter of zone of inhibition in mm (Mean ± SD), Values with different superscripts in the same column differ significantly (P < 0.05). (-) No Activity, DCM- Dichloromethane, EA– Ethyl acetate.*

**Table 4: Antibacterial activity of different solvent extracts of test plants against *P.aeruginosa* at various concentration.**

| Test plants | Test extract | Concentration (mg / 100 µl) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 10 | 20 | 30 | 40 | 50 | 60 |
| *Eucalyptus citriodora* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | - | - | - | - |
| | EA | - | - | - | 2.2±0.4$^{e}$ | 2.9±0.3$^{g}$ | 4.3±0.1$^{g}$ | 4.8±0.2$^{g}$ | 5.7±0.7$^{i}$ |
| | Ethanol | - | - | - | 2.5±0.3$^{e}$ | 4.6±0.1$^{e}$ | 5.1±0.1$^{f}$ | 5.6±0.4$^{f}$ | 8.3±0.4$^{f}$ |
| | Methanol | - | 5.5±0.1$^{a}$ | 6.5±0.3$^{b}$ | 8.6±0.2$^{c}$ | 9.6±0.3$^{c}$ | 11.2±0.2$^{c}$ | 14.5±0.2$^{b}$ | 15.4±0.5$^{b}$ |
| | Aqueous | - | 3.3±0.4$^{c}$ | 9.7±0.1$^{a}$ | 10.3±0.3$^{b}$ | 12.4±0.3$^{b}$ | 12.7±0.2$^{b}$ | 13.7±0.5$^{b}$ | 14.6±0.4$^{c}$ |
| *Ocimum sanctum* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | - | - | - | - |
| | EA | - | - | - | - | - | - | - | - |
| | Ethanol | - | - | - | - | - | 4.1±0.4$^{g}$ | 5.6±0.3$^{fg}$ | 6.6±0.2$^{h}$ |
| | Methanol | - | - | - | | | | 7.5±0.4$^{e}$ | 9.4±0.6$^{e}$ |
| | Aqueous | - | - | - | 3.5±0.3$^{d}$ | 6.6±0.3$^{d}$ | 8.4±0.2$^{d}$ | 10.3±0.2$^{c}$ | 11.6±0.1$^{d}$ |
| *Allium sativum* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | 3.3±0.1$^{f}$ | 4.4±0.6$^{g}$ | 5.9±0.3$^{f}$ | 6.6±0.1$^{h}$ |
| | EA | - | 4.4±0.3$^{b}$ | 5.1±0.3$^{c}$ | 14.3±0.3$^{a}$ | 16.3±0.3$^{a}$ | 18.0±0.1$^{a}$ | 21.5±0.3$^{a}$ | 22.6±0.7$^{a}$ |
| | Ethanol | - | - | - | 3.5±0.7$^{d}$ | 6.4±0.3$^{d}$ | 7.5±0.3$^{e}$ | 8.8±0.1$^{d}$ | 11.5±0.4$^{d}$ |
| | Methanol | - | - | - | - | - | - | 2.4±0.6$^{h}$ | 4.5±0.6$^{j}$ |
| | Aqueous | - | - | - | - | 1.8±0.2$^{h}$ | 3.5±0.2$^{h}$ | 5.8±0.3$^{f}$ | 7.1±0.3$^{g}$ |
| **Streptomycin (50 µg / 100 µl)** | | **15.3 ± 0.2** | | | | | | | |
| **Acetone : DMSO(1:1v / v)** | | - | | | | | | | |

*Values are diameter of zone of inhibition in mm (Mean ± SD). Values with different superscripts in the same column differ significantly (P < 0.05). (-) No Activity, DCM- Dichloromethane, EA–Ethyl acetate.*

Methanol extract of *E.citriodora* exhibited higher effect against *P.aeruginosa* and *E.coli* at the concentration of 1 mg / 100 µl and aqueous extract of *E.citriodora* and ethyl acetate extract

of *A.sativum* were also able to inhibit the growth of *P.aeruginosa* at this concentration but 0.5 mg/ 100 µl of all test extracts of all three test plants failed to inhibit the growth of *P.aeruginosa* and *E.coli*. Hexane and DCM extract of *E.citriodora* and *O.sanctum*, ethyl acetate extract of *O.sanctum* and hexane extract of *A.sativum* had no inhibition on *P.aeruginosa* even at the highest test concentration. Ethanol, methanol and aqueous extracts of *O.sanctum* and DCM, ethyl acetate, ethanol and aqueous extracts of *A.sativum* and aqueous extract of *E.citriodora* were also able to inhibit the growth of *E.coli* at concentration range from 10 to 50 mg / 100 µl and other solvent extracts of these three test plants had no effect on *E.coli* **(Table 4 and 5**). The standard antibiotic streptomycin produced higher inhibitory effect on *B.subtilis* and followed by *P.aeruginosa* and *S.aureus*. *E.coli* was found to be least sensitive to streptomycin (**Table 2-5**).

**Table 5: Antibacterial activity of different solvent extracts of test plants against *E.coli* at various concentration.**

| Test plants | Test extract | Concentration (mg / 100 µl) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **0.5** | **1** | **10** | **20** | **30** | **40** | **50** | **60** |
| *Eucalyptus citriodora* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | - | - | - | - |
| | EA | - | - | - | - | - | - | - | - |
| | Ethanol | - | - | - | - | - | - | - | - |
| | Methanol | - | 3.1±0.3 | 7.0±0.2$^{a}$ | 8.3±0.3$^{a}$ | 9.4±0.2$^{a}$ | 11.8±0.8$^{a}$ | 14.3±0.2$^{a}$ | 16.0±0.6$^{b}$ |
| | Aqueous | - | - | 2.5±0.4$^{c}$ | 3.4±0.1$^{c}$ | 7.3±0.2$^{b}$ | 8.3±0.2$^{d}$ | 9.6±0.2$^{c}$ | 10.5±0.5$^{e}$ |
| *Ocimum sanctum* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | - | - | - | - |
| | EA | - | - | - | - | - | - | - | - |
| | Ethanol | - | - | - | - | - | 10.7±0.2$^{b}$ | 14.5±0.1$^{a}$ | 16.7±0.7$^{a}$ |
| | Methanol | - | - | - | | - | | 8.7±0.3$^{d}$ | 11.3±0.6$^{d}$ |
| | Aqueous | - | - | - | - | 3.2±0.2$^{c}$ | 4.2±0.2$^{e}$ | 8.6±0.2$^{d}$ | 10.3±0.5$^{e}$ |
| *Allium sativum* | Hexane | - | - | - | - | - | - | - | - |
| | DCM | - | - | - | - | - | 1.5±0.7$^{g}$ | 3.2±0.9$^{f}$ | 3.7±0.3$^{g}$ |
| | EA | - | - | 3.2±0.6$^{b}$ | 5.0±0.4$^{b}$ | 7.2±0.2$^{b}$ | 9.3±0.4$^{c}$ | 10.4±1.1$^{b}$ | 12.9±0.7$^{c}$ |
| | Ethanol | - | - | - | - | 1.7±0.4$^{d}$ | 2.7±0.1$^{f}$ | 4.3±0.2$^{e}$ | 8.4±0.4$^{f}$ |
| | Methanol | - | - | - | - | - | - | - | - |
| | Aqueous | - | - | - | - | - | - | 2.4±0.7$^{g}$ | 4.0±0.3$^{g}$ |
| **Streptomycin (50 µg / 100 µl)** | | **3.0 ± 0.1** | | | | | | | |
| **Acetone : DMSO(1:1v / v)** | | – | | | | | | | |

*Values are diameter of zone of inhibition in mm (Mean ± SD). Values with different superscripts in the same column differ significantly (P < 0.05). (-) No Activity, DCM- Dichloromethane, EA*

## Phytochemical screening

All test phytochemicals were detected in different solvent extracts of all the test plants, but tannin was not detected in any test extracts of *A.sativum* (Table 6). Ethyl acetate and ethanol extracts of *E.citriodora* possessed all the test phytochemicals except flavonoids. Ethanol, methanol and aqueous extracts of *A.sativum* were found to have cardiac glycosides, saponins and terpenoids. All the test extracts of *O.sanctum* except hexane extract possessed at least one of the test phytochemicals (Table 6).

## DISCUSSION

In order to evaluate the antibacterial activity of medicinal plants different extractions and bioassay techniques are employed [16, 17]. In general, the solvent system used for the extraction plays a significant role in the solubility of the active principles of plant materials which in turn it influences on the antibacterial activities of the extracts [17]. In the present study sequential extraction was carried out with different solvents from low polar to high polar. Higher yield was obtained in high polar solvents than low polar solvents. This indicates that the test plant materials were found to possess number of polar compounds than non polar compounds [18]. It was pointed out that sequential extraction of plant material with solvents of various polarities is useful to extract the biologically active constituents from plants and also a favorable technique for further study of plant extracts [19].

In an earlier study it was pointed out that the hot aqueous extract of bulb of *A.sativum* did not

show inhibitory effect on *S.aureus* and *E.coli* by disc diffusion assay [20]. Another study reveled that direct heat aqueous extracts of whole plant of *A.sativum* failed to inhibit the growth of *S.aureus* and *P.aeruginosa* by disc diffusion method [21]. In the present study, aqueous extract of *A.sativum* was able to inhibit the growth of all above three bacteria (**Table 3 - 5**) by well diffusion assay.

**Table 6: The presence of phytochemicals in the different solvent extracts of test plants.**

| Test plants | Solvent used | Presence of Phyotychemicals | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tannins | Alkaloids | Cardiac glycoside | Saponins | Flavonoids | Terpenoids |
| *Eucalyptus citriodora* | Hexane | – | – | – | – | – | – |
| | DCM | + | + | + | – | – | + |
| | EA | + | + | + | + | – | + |
| | Ethanol | + | + | + | + | – | + |
| | Methanol | – | – | + | + | + | – |
| | Aqueous | + | + | + | + | – | – |
| *Ocimum sanctum* | Hexane | – | – | – | – | – | – |
| | DCM | – | – | – | – | – | + |
| | EA | + | – | + | – | – | – |
| | Ethanol | + | + | + | – | + | – |
| | Methanol | + | – | – | – | + | – |
| | Aqueous | – | – | + | + | + | – |
| *Allium sativum* | Hexane | – | – | + | – | – | – |
| | DCM | – | – | + | – | – | + |
| | EA | – | + | + | – | + | + |
| | Ethanol | – | – | + | + | – | + |
| | Methanol | – | + | + | + | – | + |
| | Aqueous | – | – | + | + | – | + |

*(+) Presence of phytochemical (-) Absence of phytochemical*

The variation of results between former and present study may be due to different method of extraction or assay method or both. In both of the earlier studies extraction was carried out using hot water. Therefore, the absence of inhibitory effect may be due to the inactivation of active principles that may be thermo labile. It has been also reported that the allicin, the biologically active phytochmical of *A.sativum* is not stable at higher temperature [22]. In another study of direct cold aqueous extract of bulb of *A.sativum* (5mg) demonstrated that the growth of *S.aureus* was inhibited by both agar well diffusion and disc diffusion methods [23]. Therefore it can be concluded that the cold extraction method may be an effective method for the extraction of garlic. However, in the present study absence of the inhibitory effect of aqueous extract of *A.sativum* on *B.subtilis* may be due to the absence of active constituents which would have been extracted out in methanol before the aqueous extraction.It was found that cold ethanol extract of *A.sativum* failed to inhibit the growth of *S.aureus* and *E.coli* when tested by disc diffusion method [20]. It was also reported that the cold methanol extract of whole plant of *A.sativum* had no effect on *P.aeruginosa* and *S.aureus* when tested by well diffusion method [21]. But in the present study the above bacteria were inhibited by the respective extracts.

A former study showed that the direct ethanol extract of leaf of *O.sanctum* (8 mg) failed to inhibit the growth of *E.coli* [24]. In another instance it reveled that the direct methanol extract of *O.sanctum* did not inhibited the growth of *E.coli* and *P.aeruginosa* [25]. In the present study the ethanol and methanol extracts of *O.sanctum* had the ability to inhibit the growth of all test bacteria including *E.coli* and *P.aeruginosa*.

In the previous cases direct ethanol and methanol extracts of *A.sativum* and *O.sanctum* were used but in the present study the respective extracts were obtained through sequential extraction method. Abubakar (2009) found that sequentially extracted ethanol extract of bulb of *A.sativum* (500 µl of 50 mg / ml) was able to inhibit the growth of *S.aureus*, *P.aeruginosa* and *E.coli* by agar well diffusion method [17].

Therefore differences in the effects between previous and present studies might be due to the different extraction method. In single solvent extraction, the extracts may have numerous compounds but the sequentially extracted solvents extracts may have less number of compounds as compounds are dissolved in solvents according to their polarity. Therefore, the antagonistic effect will be at low level in the solvent extracts obtained by sequential extraction. It was reported that presence of numerous compounds can lead to antagonistic effect [26]. Also in the sequential extraction method partial separation of compounds will facilitate further isolation and purification of active compounds. In a former study ethanol extract of *E.citriodora* (10 g / 100 ml) had the ability to inhibit the growth of *P.aeruginosa* and *S.aureus* when tested by agar well diffusion method whereas aqueous extract failed to inhibit the growth of above two bacteria by agar disc diffusion method [21]. In the present study both of above bacteria were inhibited by both ethanol and aqueous extracts at various concentrations (**Table 3 and 4**). It further supports that sequential extraction would be more effective than single solvent extraction.

It has been reported that single solvent extract of aqueous and ethanol of *A.sativum* possessed flavonoids, alkaloids and triterpenoids [27]. It was also found the presence of alkaloids and flavanoids in alcoholic extract of bulb of *A.sativum* [28]. In the present study sequential extracts of different solvents of *A.sativum* showed presence of above phytochemicals and also in addition these extracts had cardiac glycosides and saponins.

One of the previous studies indicated that the alkaloids, glycosides, terpenoids and tannins were detected in ethanol extract of *O.sanctum* [29]. Another phytochemicals study reported that whole part of *O.sanctum* possessed cardiac glycoside, and tannins [28]. Methanol extracts of *O.sanctum* possessed glycosides, and saponins [30]. In the present study except hexane extract all other extracts of *O.sanctum* found to have at least one of the above phytochemicals and also in addition flavonoids was detected in ethanol, methanol and aqueous extracts (Table 6). It was also noted that leaf of *O.gratissimum* was found to have flavonoids [31, 32]. Previous study reported that volatile oil of *E.citridora* inhibited the growth of *E.coli* and *S.aureus* but no effect on *P.aeruginosa* using agar well diffusion method [33]. The present study revealed that all solvent extracts of *E.citriodora* was found to have effect on *S.aureus* at various concentration. Methanol and aqueous extract of *E.citriodora* had inhibitory effect on *E. coli* and *P. aeruginosa* and ethyl acetate and ethanol extracts of *E. citriodora* had effect against *P. aeruginosa* (**Table 2-5**). The varying results of previous and present studies may be due to the different extraction methods employed in both study.

## CONCLUSION

Present preliminary *in vitro* antibacterial screening and phytochemical analysis of *Ocimum sanctum, Eucalyptus citriodora* and *Allium sativum* revealed that ethyl acetate, ethanol, methanol and aqueous extracts of *Eucalyptus citriodora*, ethyl acetate extract of *Allium sativum* and ethyl acetate and aqueous extract of *Ocimum sanctum* were found to be effective for the growth control of test bacteria. Results of phytochemicals study further provide the information regarding to the activity of these test extracts. Therefore, these extracts could be used for further isolation and purification of active compounds.

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