# A STUDY OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL STUDIES OF SELECTED MEDICINAL PLANTS

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**Abstract: Medicinal plants have effective antimicrobial potential. In the present study,the leaves of five medicinal plants have been selected for screening of phytochemical and antibacterial efficiencies in different extracts (Ethanol, Acetone and Aqueous). The extract of *Vitex negundo, Phyllanthus niruri, Swertia chirata, Adhatoda vasica* and *Ocimum sanctum* were tested against a gram positive bacteria, *Staphylococcus aureus* and a gram negative bacteria, *Escherichia coli.* Among the selected plants, aqueous and alcoholic extracts of *Swertia chirata*, displayed highest antimicrobial zone against *S.aureus. E.coli* and *S.aureus were highly sensitive* to the combined effects aqueous extracts of *Swertia chirata and* acetone extract of *Ocimum sanctum* . The qualitative analysis of different phytochemicals such as Alkaloids, Flavanoids, Saponins Tannins and Quinones are tested in the extracts of the selected medicinal plants Alkaloids were present in all the extracts of all the selected medicinal plants.**

**Key words: medicinal plants, antibacterial activity, phytochemicals, *Vitex negundo, Phyllanthus niruri, Swertia chirata, Adhatoda vasica, Ocimum sanctum, Escherichia coli, Staphylococcus aureus***

## I. INTRODUCTION

The plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now days, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination [3].

The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [4]. Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well [4].Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world [12,23]. Thus, the present study deals with the screening of phytochemical tests and antibacterial efficiency against gram positive bacteria, *Staphylococcus aureus* and a gram negative bacteria, *Escherichia coli* of five medicinal plants viz. *Vitex negundo, Phyllanthus niruri, Swertia chirata, Adhatoda vasica, Ocimum sanctum.*

## II. MATERIALS AND METHODS

### *MATERIALS*

#### *1. Plants Employed For The Study*

Leaves of five medicinal plants viz *Vitex negundo, Phyllanthus niruri, Swertia chirata, Adhatoda vasica* and *Ocimum sanctum*.

#### 2. *Organisms Used For The Study*

The organisms used for the present study were *Staphylococcus aureus* and *Escherichia coli*.

#### 3. *Preparation of Extracts*

Leaves of selected plants were pulverized and extracted twice in water, acetone and ethanol (1:10 w/v) at room temperature for 48 hrs and filtered. The filtrates were concentrated to dryness under reduced conditions at room temperature.

***METHODS***

***1 ANTIMICROBIAL STUDIES***

*a) DIRECT CONTACT METHOD*

In the direct contact method, sterile swabs soaked in bacterial culture (in pepton water) were applied over a dry nutrient agar plate. Approximately equal amount of the crude material were placed over the lawn culture at different positions with sterile forceps. The plates were then incubated at 37°C overnight. Then the zone of inhibition around the material was used for the preliminary screening of the medicinal plants for their antibacterial activity.

*b) DISC DIFFUSION METHOD*

This method was employed for water extract, acetone extract, and ethyl alcohol extract. Lawn culture of the bacteria was prepared. In case of water extract sterile filter paper disc made of Whatmann filter paper No: 1 was dipped in the supernatant, excess supernatant was wiped off and placed over the lawn culture at different positions. In the case of acetone, ether and ethyl alcohol extracts, filter paper discs were spread on a sterile petri plate and then the supernatant was applied on the disc using a pasteur pipette. The solvent was allowed to evaporate after which they were placed over the lawn culture with sterile forceps and incubated at 37 °C overnight.

***2 PHYTOCHEMICAL STUDIES***

*i) TEST FOR ALKALOIDS:*

The extract was mixed with 2 ml of Wagner's reagent. Reddish brown color precipitate indicates the presence of alkaloids.

*ii) TEST FOR FLAVONOIDS:*

Small quantity of the test solution was treated with a few drops of lead acetate solution. The formation of yellow color or yellow cream color indicates the presence of flavonoids.

*iii) TEST FOR SAPONINS:*

The extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

*iv) TEST FOR TANNINS:*

Treat the sample solution with few drops of Ferric Chloride solution. Presence of green-blue or blue-black colour indicates the presence of tannin.

*v) TEST FOR QUINONES:*

Dilute NaOH was added to 1 ml of crude extract. Blue-green or red colouration indicates the presence of quinones.

## III.RESULTS AND DISCUSSION

Result obtained in the present study revealed that tested leaf extracts possess antibacterial activity against both *E.coli* and *S.aureus*. Among the five medicinal plants, aqueous extract of *Swertia chirata* displayed highest antibacterial zone against *S.aureus*. The both *S.aureus* and *E.coli*were sensitive to all other selected plants. Based on the results the extract which showed maximum inhibition against bacteria was selected and a combination of these extracts were studied for their antibacterial activity against *E.coli* and *S.aureus.* The combined effects of aqueous extracts of *Swertia chirata and* acetone extract of *Ocimum sanctum* showed antibacterial zone of 31mm against *E.coli* and 38mm antibacterial zone against *S.aureus*.

The qualitative analysis of different phytochemicals such as Alkaloids, Flavanoids, Saponins Tannins and Quinones are tested in the extracts of the selected medicinal plants. Alkaloids were present in all the extracts of selected medicinal plants. Based on the result it can be study concluded that the presence of phytochemicals which influenced the antibacterial activity.

***1. ANTIMICROBIAL STUDIES***

| EXTRACTS | *Vitex negundo* | *Phyllanthus niruri* | *Swertia chirata* | *Adhatoda vasica* | *Ocimum sanctum* |
|---|---|---|---|---|---|
| **Water** | 14mm | 14mm | 23mm | 14mm | 17mm |
| **Acetone** | 13mm | 14mm | 14mm | 19mm | 15mm |
| **Alcohol** | 13mm | 15mm | 20mm | 11mm | 18mm |

TABLE 1: **Antibacterial Activity Against *E.coli***

| EXTRACTS | *Vitex negundo* | *Phyllanthus niruri* | *Swertia chirata* | *Adhatoda vasica* | *Ocimum sanctum* |
|---|---|---|---|---|---|
| **Water** | 19mm | 13mm | 28mm | 11mm | 17mm |

| | | | | | |
|---|---|---|---|---|---|
| **Acetone** | 15mm | 18mm | 22mm | 18mm | 25mm |
| **Alcohol** | 16mm | 17mm | 25mm | 13mm | 18mm |

TABLE 2: **Antibacterial Activity Against *S.auerus***

Based on the above results the extract which showed maximum inhibition against bacteria was selected and a combination of these extracts were studied for their antibacterial activity against *E.coli* and *S.aureus*

| Aqueous extract of *Swertia chirata* and Acetone extract of *Ocimum sanctum* | *E.coli* | *S.auerus* |
|---|---|---|
| | 31mm | 38mm |

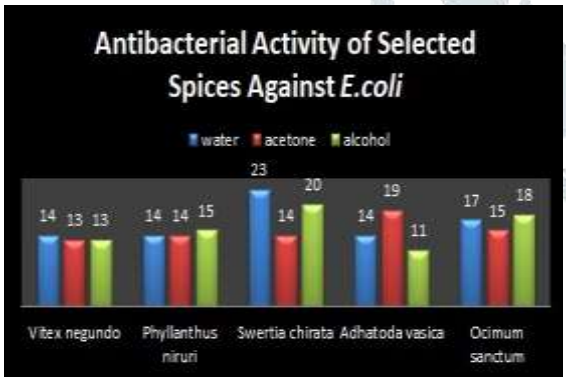
TABLE 3: **The combination effect of Aqueous extract of *Swertia chirata* and Acetone extract of *Ocimum sanctum* against *E.coli & S.auerus.***

### *2. PHYTOCHEMICAL STUDIES*

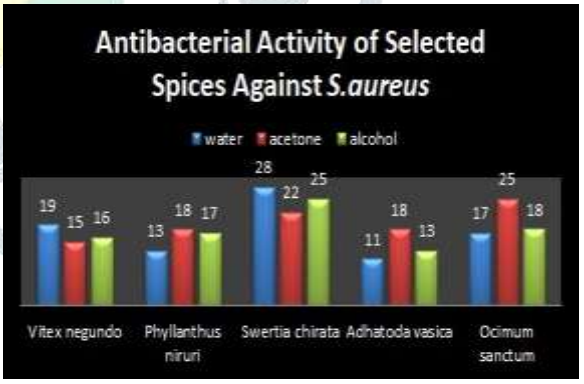
The present study revealed that tested leaf extract possess the different phytochemicals such as Alkaloids, Flavanoids, Saponins Tannins and Quinones are tested in the extracts of the selected medicinal plants. Alkaloids were present in all the extracts of selected plants.

| **SL. NO** | **Chemical Constituents** | ***Vitex negundo*** | | | ***Phyllanthus niruri*** | | | ***Swertia chirata*** | | | ***Adhatoda vasica*** | | | ***Ocimum sanctum*** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Water** | **Acetone** | **Alcohol** | **Water** | **Acetone** | **Alcohol** | **Water** | **Acetone** | **Alcohol** | **Water** | **Acetone** | **Alcohol** | **Water** | **Acetone** | **Alcohol** |
| **1** | **Alkaloids** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **2** | **Flavanoids** | + | - | + | - | + | + | + | - | - | + | - | - | + | + | + |
| **3** | **Saponins** | + | - | + | + | + | + | + | + | - | + | - | + | + | - | + |
| **4** | **Tannins** | + | + | + | + | + | + | + | + | + | - | - | - | + | + | + |
| **5** | **Quinones** | + | + | - | + | - | - | - | + | + | - | + | + | + | + | - |

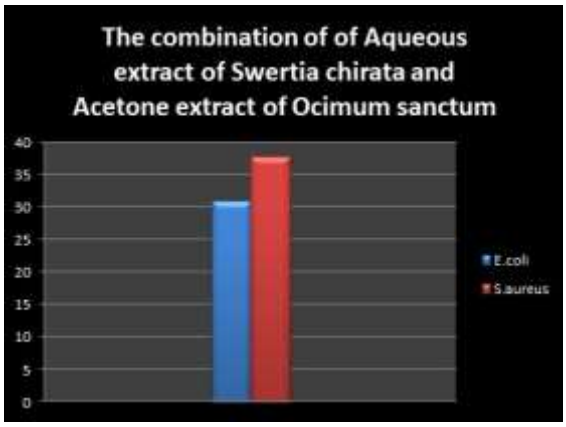
TABLE 4: **Phytochemical analysis of selected samples.**



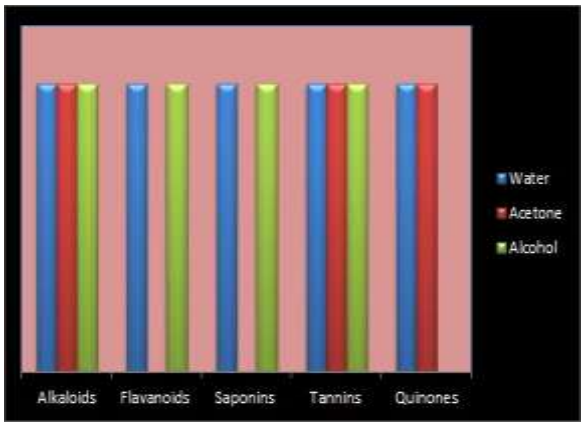

GRAPH 1: Antibacterial Activity against *E.coli*



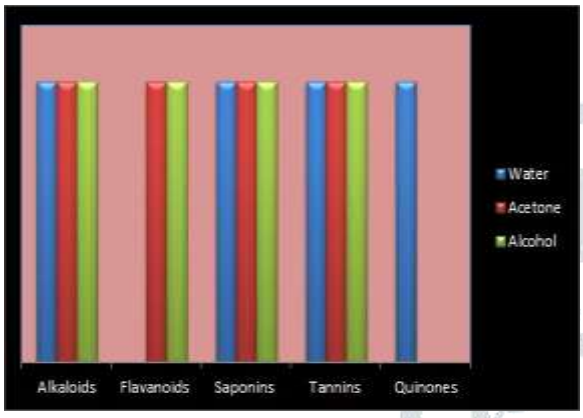

GRAPH 2: Antibacterial Activity against *S.aureus*



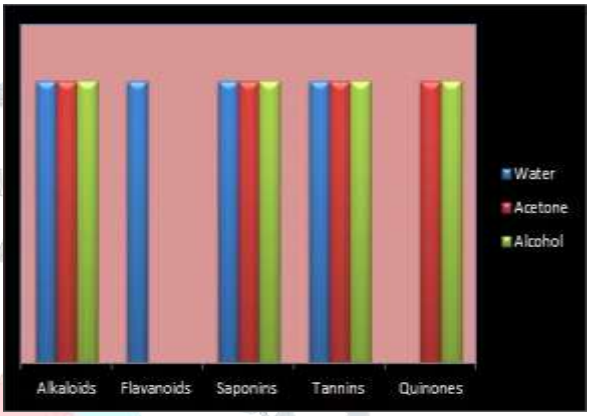

Combination effects



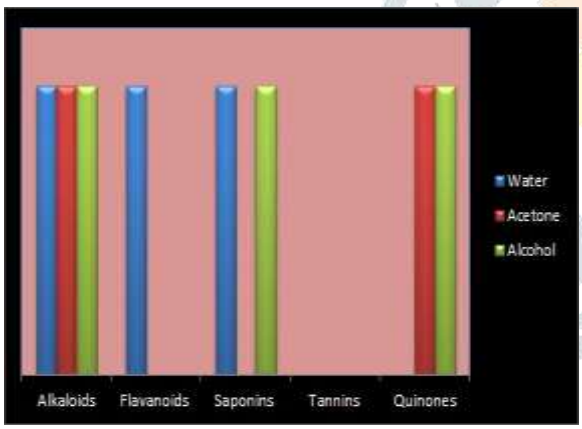

GRAPH 3: GRAPH 4: Phytochemical analysis of *Vitex negundo*



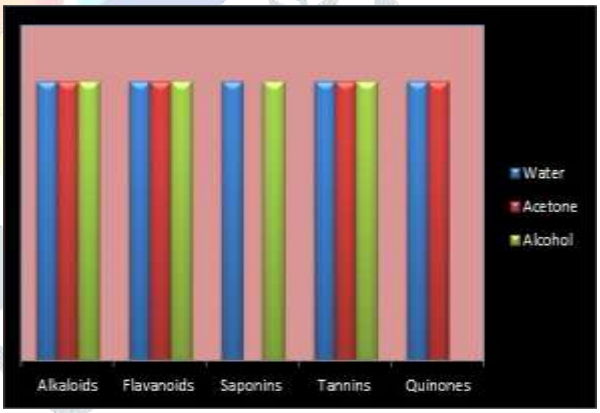

Phytochemical analysis of *Phyllanthus niruri*




GRAPH 5: GRAPH 6: Phytochemical analysis of *Swertia chirata*




GRAPH 7: Phytochemical analysis of *Adatoda vasica*




GRAPH 8: Phytochemical analysis of *Ocimum sanctum*


FIG 1: Control

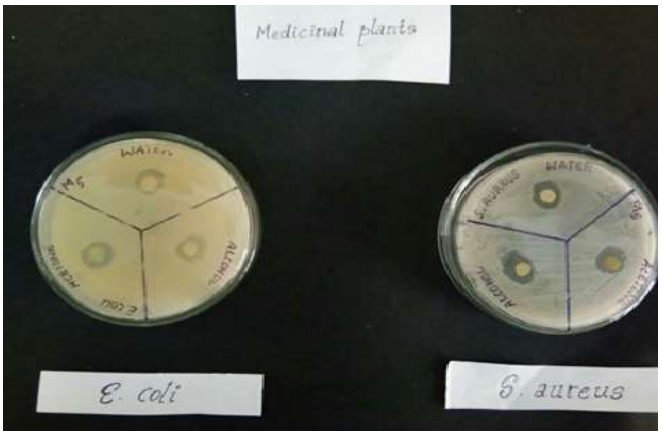
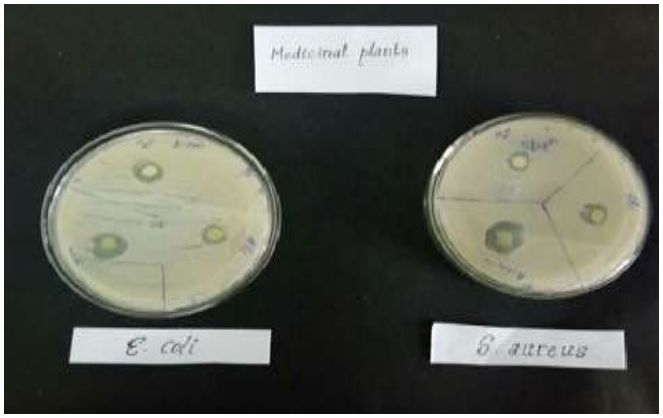



FIG 2: Antibacterial Activity of *Vitex negundo*




Antibacterial Activity of *Phyllanthus niruri*

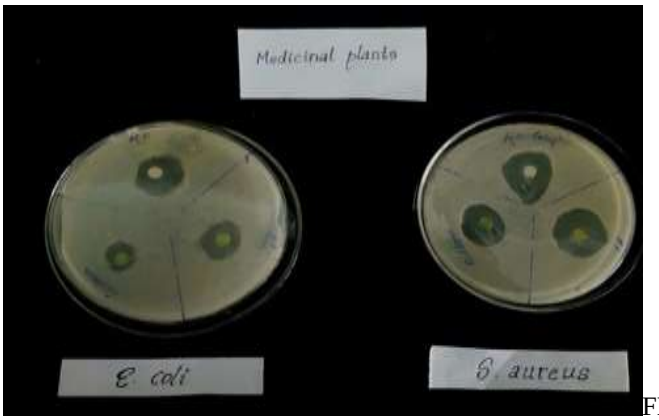



FIG 3:

FIG 4: Antibacterial Activity of *Swertia chirata*


FIG 5: Antibacterial Activity of *Adatoda vasica*

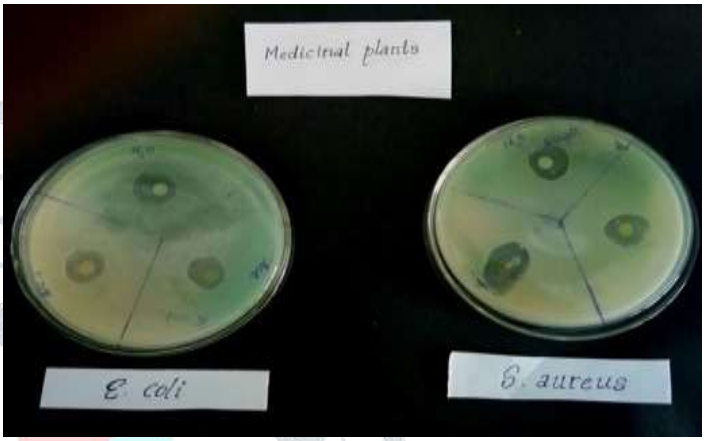



FIG 6: Antibacterial Activity of Ocimum sanctum

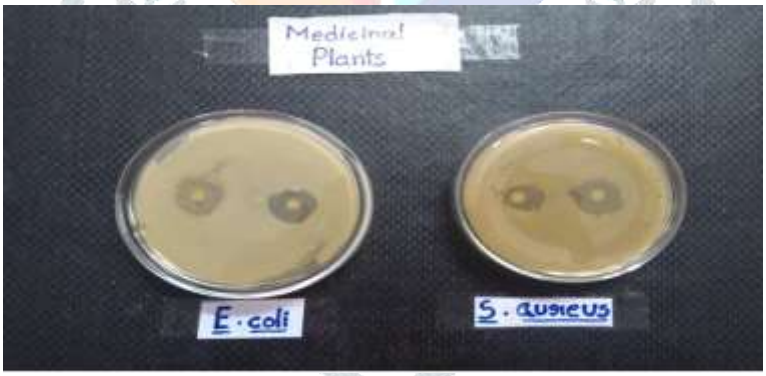



FIG 7: Antibacterial Activity of Combination of Medicinal plants

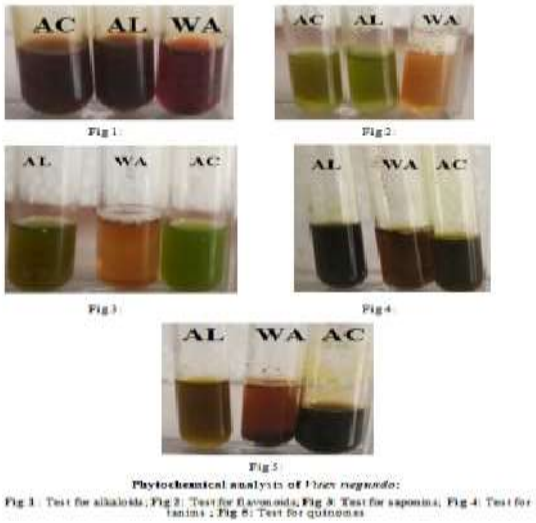



FIG 8: Phytochemical analysis of *Vitex negundo*

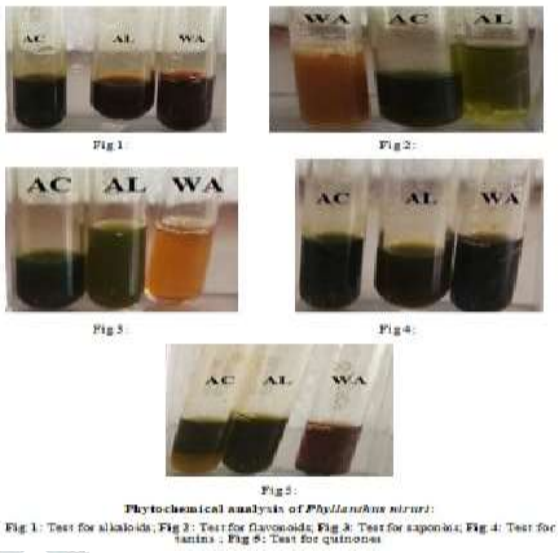



FIG 9: Phytochemical analysis of *Phyllanthus niruri*

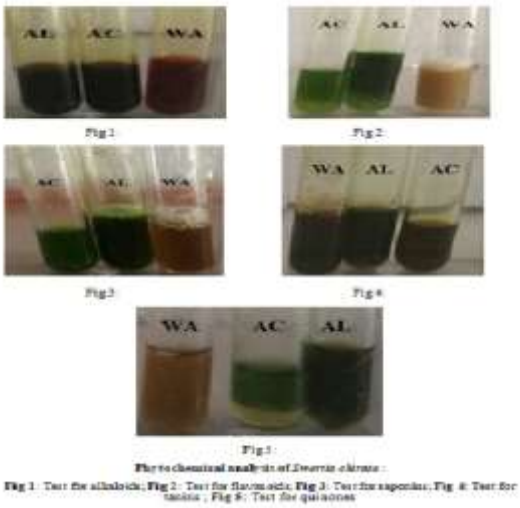



FIG 10: Phytochemical analysis of *Swertia chirata*

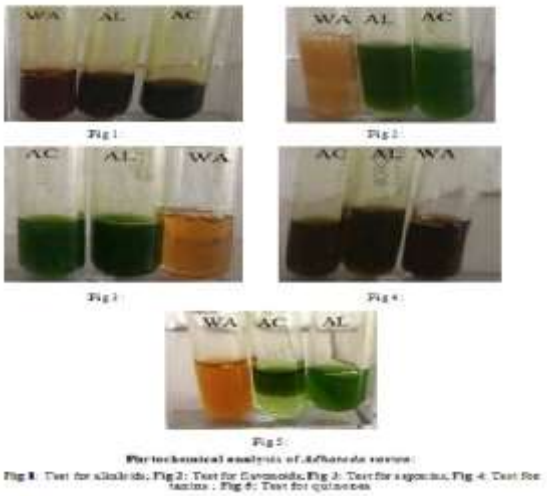



FIG 11: Phytochemical analysis of *Adatoda vasica*

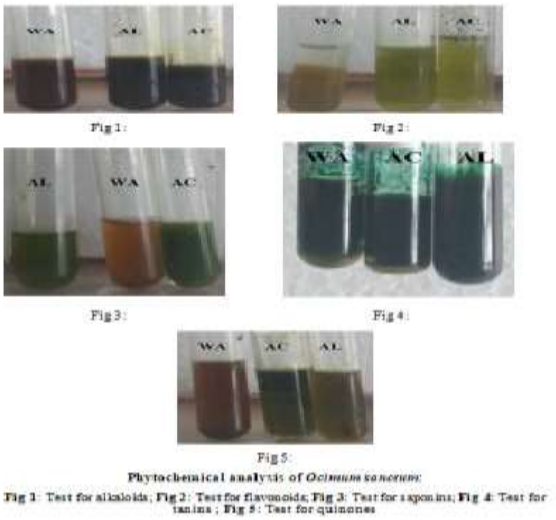



FIG 12: Phytochemical analysis of *Ocimum sanctum*

The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment[2]. The phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. In the present study, aqueous leaf extracts of *Swertia chirata* showed maximum inhibition zone against *S.aureus* as reported by Balaraju *et.al* (2008). Alcohol extracts of *Swertia chirata* showed antibacterial zone (25mm) against *S.aureus* which is correlated the studies of Kabita Nayak (2015). Alkaloids were present in all the extracts of selected plants and flavanoids were present mostly in acetone and ethanol extracts. The tannins were present in ethanol and aqueous extracts. Saponins were present in water, rarely presents in alcohol extract same as reported by Levy et al. (2004). The presence of alkaloids showed the antimicrobial activity (Rukha aminta et al, 2002). For the mentioned reasons the study concluded that the presence of phytochemicals which favored the antimicrobial activity.

## IV.CONCLUSION

Antibacterial activity and phytochemical studies of different extracts (Ethanol, Acetone and Aqueous) of five selected medicinal plants were studied. The extract of *Vitex negundo, Phyllanthus niruri, Swertia chirata, Adhatoda vasica* and *Ocimum sanctum* were tested against a gram positive bacteria, *Staphylococcus aureus* and a gram negative bacteria, *Escherichia coli.* Among the five, aqueous and alcoholic extracts of *Swertia chirata*, displayed highest antimicrobial zone against *S.aureu*s. All the other medicinal plants in the present study showed the antibacterial activity against *S.aureu*s and *E.coli*. The combined effects of aqueous extracts of *Swertia chirata* and acetone extract of *Ocimum sanctum* showed antibacterial zone of 38mm against *E.coli* and 31mm antibacterial zone against *S.aureus*.

Medicinal plants are widely used to treat disease in non-industrialized societies, not least because they are far cheaper than modern medicines. Alkaloids were present in all the extracts of selected plants and flavanoids were present mostly in acetone and ethanol extracts. Tannins, Saponins and Quinones were rarely present in extracts.

Based on the results of the present study it is concluded that all the leaves of selected plants have potent antibacterial activity against gram-negative and gram-positive bacteria which might be due to the phytochemicals present in the leaves.

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