

# Comparative Study of Antimicrobial Activity of Selected Medicinal Plants Against Some Selected Bacteria

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**Abstract-** Nature provides medicinal agents, where active components often combine with inactive substances. In recent years, the antibiotic resistance of human pathogenic microorganisms has been increased; which led to search for new antimicrobial substances from medicinal plants. In the present study, the methanol and ethanol extracts of four medicinal plants were evaluated against some selected bacteria such as *Staphylococcus aureus*., *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Klebsiella pneumoniae*., *Pseudomonas fluorescens* and *proteus vulgaris*. The agar well diffusion method was used to perform the invitro anti-microbial activity. The measurements of zone of inhibition were taken and compared. The ethanolic extract exhibited greater antimicrobial activity compared to methanolic extracts. Plant extracts with antimicrobial properties, have potential therapeutic uses.

**Key words-** Medicinal plants, Bacterial culture, ethanol and methanol extracts, antimicrobial activity.

## I. Introduction

Tirumala and Tirupati, known as the 7 hills of Lord Venkateshwara, are located at 79°23' E longitude and are between 13°37' N to 13°43' N latitude in Chittoor District of Andhra Pradesh. The mythological and scientific studies revealed that a number of ethno medicinal plants are hidden in the hills of Tirumala. Plants provide valuable active ingredients for disease management. Additionally, it is noted that the active components of herbal remedies can be effectively combined with numerous other substances that appear to be inactive. Antibiotic resistance of human pathogenic microorganisms increased in recent years; this has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Screening plant families for antibacterial properties is crucial for discovering new therapeutic agents. The phytochemical and health benefits of numerous medicinal plants are yet to be fully discovered offering a promising future [1]. Many medicinal drugs are derived from certain plants with proven therapeutic benefits [2]. Traditional medicinal plants are often the preferred choice in developing regions due their low cost and limited availability of synthetic alternatives [3].

## Medicinal plants used in the present study

### *Tinospora cordifolia* (Guduchi)

Guduchi is the common name for *Tinospora cordifolia*, which is categorized as a large, glabrous, deciduous climbing shrub of the family Menispermaceae [4]. It's commonly used in folk and Ayurvedic medicine. The root, stem and entire plant contains chemical components such as glycosides, diterpenoid lactones, sesquiterpenoids, steroids, aliphatic compounds, phenolics, essential oils, polysaccharides, a mixture of fatty acids [8]. As it contains bioactive components and numerous therapeutic properties it can be used to treat jaundice, rheumatism, urinary diseases, skin conditions, diabetes, anemia, inflammation, allergic condition, anti-periodic qualities, radioprotective properties [5]. The root acts as an emetic and treats intestinal obstruction. Its starch is effective for chronic fever and to increase energy and appetite [5]. Used in the treatment of helminthiasis, cardiovascular illness, leprosy, rheumatoid arthritis and other disorders [5]. The plant enhances immunity and body resistance to infections when used in Ayurvedic "Rasayanas". Used to treat gastrointestinal problems like hyperacidity, colitis, worm infestations, anorexia, stomach pain, excessive thirst, vomiting and hepatitis [6,7].

### *Rubia Cordifolia* (Manjistha)

*R. cordifolia* is commonly known as "Manjistha" or Indian madder or simply Madder. *R. cordifolia* is very common at high altitudes and its dried samples are sold in the market. Both kapha and pitta are pacified by Manjistha, which is bitter, astringent, sweet in taste and heavy, dry and hot in effect. Studies reveal its rich in compounds like anthraquinones and its glycosides, naphthoquinones and its glycosides, bicyclic hexapeptides, triterpenoids and polysaccharides with diverse effects: neuroprotective, anti-tumor, antibacterial, anti-inflammatory, anti-oxidant and immunosuppressive activities. It is used to treat conditions like like gout, glandular swellings, skin infections and other disorders like pigmentation leucoderma, acne, pimples and discoloration. It is also used to treat gynecological issues like leukorrhea and menstrual irregularities. It is used as natural dye for hair, food and fabrics [9].

### *Saussurea lappa* (Kushta)

*Saussurea lappa*, or Kuth root, is a potent herb in traditional medicine, rich in phytochemicals like costunolide and cynaropicrin, which show bioactive potential for developing new molecules. They have medicinal uses like anti-ulcer, anti-convulsant, anti-

cancer, hepatoprotective, anti-arthritis, anti-viral activities [10]. It treats sore throat, tonsillitis, pleurisy, rheumatoid arthritis, typhoid fever and many of bacterial and fungal infections. Due to the significant proven activities, it is having considerable chance for new drug discovery [10]. *Saussurea lappa* root extracts exhibit antioxidant, antibacterial, and antifungal properties. [11].

**Glycyrrhiza glabra (Yastimadhu)**

Glycyrrhiza glabra, or licorice, is a centuries-old herb used in food and medicine, with roots containing a compound 50 times sweeter than sugar. The compounds are used against allergies, bronchitis, colds, sore throats, tuberculosis and stomach problems including stomach ulcers, heartburn from reflux, inflammatory disorders, skin diseases, stress relief and liver problems [12].

The extracts are utilized in the pharmaceutical and food industries. The root makes a thirst-quenching tea and serves as a natural tooth cleanser when dried. *G. glabra* is used industrially to produce food flavorings and sweeteners. The root is used as a flavouring agent in chewing gum, candies, baked goods, ice cream, and soft drinks. The root extracts act as foaming agents in beer and fire extinguishers. The leftover root fibers are then repurposed for insulation, wallboard and boxboard. *G. glabra* is used in cosmetics for skin pigmentation in topical products and is being ingredient in topical products for that purpose [12].

S. No	Name of Plant	Common Name in Telugu	English Name	Sanskrit Name	Hindi Name	Parts used	Family	Medicinal Properties
1	<i>Tinospora cordifolia</i>	Tippateega	Indian tinospora, Tinospora gulancha	Guduchi	Giloya Guduchi	Stem	Menispermaceae	Cure urinary tract infections, improves immune system
2	<i>Rubia cordifolia</i>	Manjista	Indian Madder	Manjista Samanga	Manjit	Bark	Rubiaceae	Antiseptic, cure ulcers, inflammation, other skin disorders
3	<i>Saussurea lappa</i>	Kostu, Chengalva-kostu.	Costus, Kutroot	Kushta	Koont, Kuth	Roots	Asteraceae	Antiseptic, disinfectant, anti-inflammatory
4	<i>Glycyrrhiza glabra</i>	Atimadhuram, Atimadhuramu Yashti-Madhukam.	Liquorice, Licorice	Yastimadhu	Jethimadhu, Multhatti	Root	Fabaceae	In the treatment of asthma, acute chronic bronchitis, mouth, gastric, duodenal ulcers

**Table 1: Details and the Medicinal values of the plants selected for the present study**



**Figure 1: Plants and their parts used in the study**

## II. Materials and Methods

### A. Collection of medicinal plant samples

The parts of different medicinal plants were collected from Tirumala and Tirupati of Chittoor District, Andhra Pradesh. They were shade dried and were ground to a fine powder using a blender.

### B. Processing of plant material

The plant parts were cleaned with tap and distilled water, dried under shade, powdered, and stored in containers.

### C. Bacterial samples:

The test bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas fluorescense*, *Proteus vulgaris* were sourced from Sri Venkateswara Institution of Medical Sciences (SVIMS), Tirupati and maintained on nutrient agar slants at 4°C.

Strain	Category of Test organism	Shape of organism
<i>Staphylococcus aureus</i>	Gram positive	Cocci
<i>Bacillus subtilis</i>		Rod
<i>Bacillus megaterium</i>		Rod
<i>Escherichia coli</i>	Gram negative	Rod
<i>Klebsiella pneumoniae</i>		Rod
<i>Pseudomonas fluorescense</i>		Rod
<i>Proteus vulgaris</i>		Rod

**Table 2: Test Organisms used in the present study**

### D. Preparation of plant extract

The ethanolic and methanolic extracts of 4 medicinal plants were prepared by dissolving 10gms of plant extract powder in 100ml ethanol and methanol separately. The mixture was shaken for 48 hours on a rotary shaker. The mixture was filtered through Whatmann filter paper and the filtrate was stored at 4°C. The filtrate was used for the antibacterial assay.

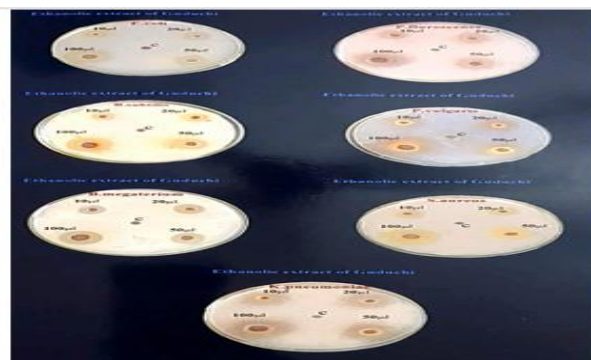
### E. Antimicrobial Activity Assay Using Agar Well Diffusion Method

20 ml of sterile Muller Hinton Agar was poured into sterile petriplate and allowed to solidify. Bacterial cultures were swabbed on plates, and wells were created with a sterile cork borer. Plant extracts concentrations (10, 20, 50 and 100µl) were added, and plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured in millimeters. A control well was made at the center of the plate in which solvent without extract was placed.

## III. Results:

### Antibacterial activity of Organic extracts of plant extracts: -

The antimicrobial activity of ethanol and methanol and extracts of four Indian medicinal plants i.e., *Tinospora cordifolia*, *Rubia cordifolia*, *Saussurea lappa*, *Glycyrrhiza glabra* were investigated by using agar well diffusion method (Figures- 2-5 and Tables 3 – 6 and the results were represented (figure 6- 9) against selected bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas fluorescense* and *Proteus vulgaris*. All the medicinal plant extracts used against the bacteria have showed diverse degree of antimicrobial activity against the pathogens.



**Figure 2: Ethanolic extract of *Tinospora cordifolia***

Organism	Type of Extract	Concentration of Extract (in $\mu$ l)			
		10	20	50	100
		Zone of Inhibition (in mm)			
<i>Escherichia coli</i>	Ethanol	4	5	6	10
	Methanol	-	-	-	-
<i>Proteus vulgaris</i>	Ethanol	8	10	11	13
	Methanol	-	-	-	-
<i>Staphylococcus aureus</i>	Ethanol	6	7	9	12
	Methanol	-	-	-	-
<i>Klebsiella pneumoniae</i>	Ethanol	10	11	12	14
	Methanol	-	-	-	-
<i>Pseudomonas fluorescense</i>	Ethanol	4	5	6	10
	Methanol	-	-	-	-
<i>Bacillus subtilis</i>	Ethanol	3	5	6	9
	Methanol	-	-	-	-
<i>Bacillus megaterium</i>	Ethanol	4	6	9	11
	Methanol	-	-	-	-

Table 3: Antibacterial Activity of Extract of *Tinospora cordifolia* on Test Organisms



Figure 3: Ethanolic extract of *Rubia cordifolia*

Organism	Type of Extract	Concentration of Extract (in $\mu$ l)			
		10	20	50	100
		Zone of Inhibition (in mm)			
<i>Escherichia coli</i>	Ethanol	3	4	6	9
	Methanol	-	-	-	-
<i>Proteus vulgaris</i>	Ethanol	3	4	6	10
	Methanol	-	-	-	-
<i>Staphylococcus aureus</i>	Ethanol	3	4	6	9
	Methanol	-	-	-	-
<i>Klebsiella pneumoniae</i>	Ethanol	5	7	8	11
	Methanol	-	-	-	-
<i>Pseudomonas fluorescense</i>	Ethanol	-	4	5	6
	Methanol	-	-	-	-
<i>Bacillus subtilis</i>	Ethanol	4	6	7	9
	Methanol	-	-	-	-
<i>Bacillus megaterium</i>	Ethanol	4	5	6	8
	Methanol	-	-	-	-

Table 4: Antibacterial Activity of Extract of *Rubia cordifolia* on Test Organisms

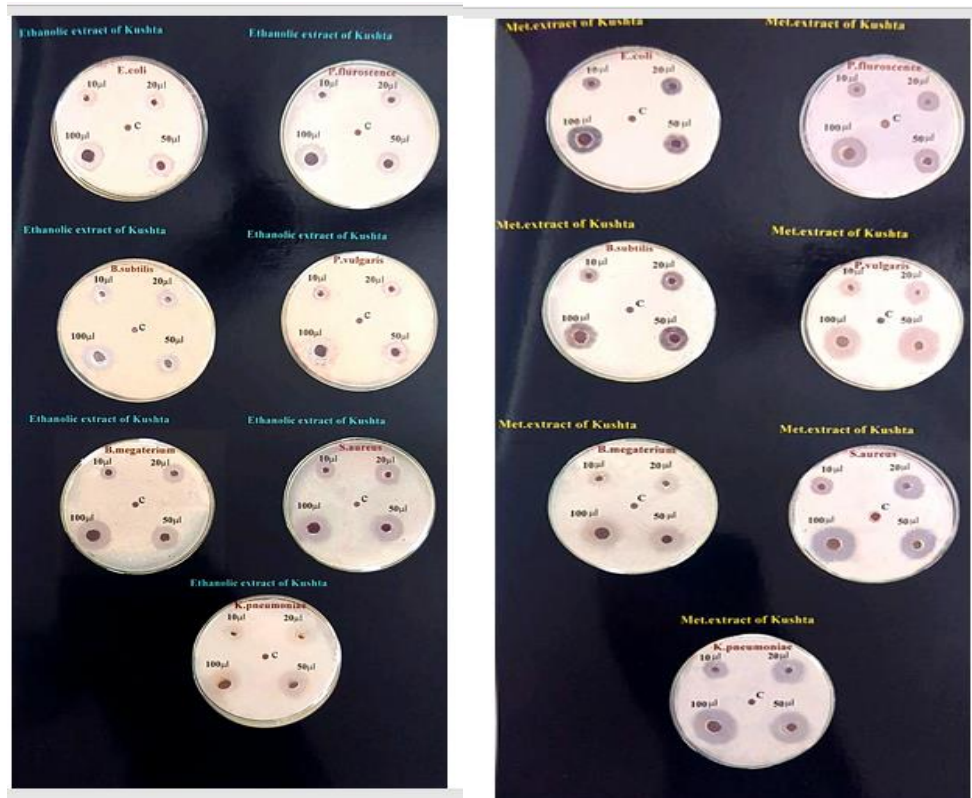


Figure 4: Ethanolic and Methanolic extract of *Saussurea lappa*

Organism	Type of Extract	Concentration of Extract (in $\mu$ l)			
		10	20	50	100
		Zone of Inhibition (in mm)			
<i>Escherichia coli</i>	Ethanol	4	4	6	7
	Methanol	3	4	5	7
<i>Proteus vulgaris</i>	Ethanol	3	4	6	7
	Methanol	4	6	9	9
<i>Staphylococcus aureus</i>	Ethanol	4	6	8	9
	Methanol	5	6	8	11
<i>Klebsiella pneumoniae</i>	Ethanol	4	7	8	9
	Methanol	5	8	9	11
<i>Pseudomonas fluorescense</i>	Ethanol	3	4	5	8
	Methanol	3	4	5	7
<i>Bacillus subtilis</i>	Ethanol	3	4	6	8
	Methanol	4	5	6	7
<i>Bacillus megaterium</i>	Ethanol	3	5	6	9
	Methanol	4	5	6	8

Table 5: Antibacterial Activity of Extract of *Saussurea lappa* on Test Organisms

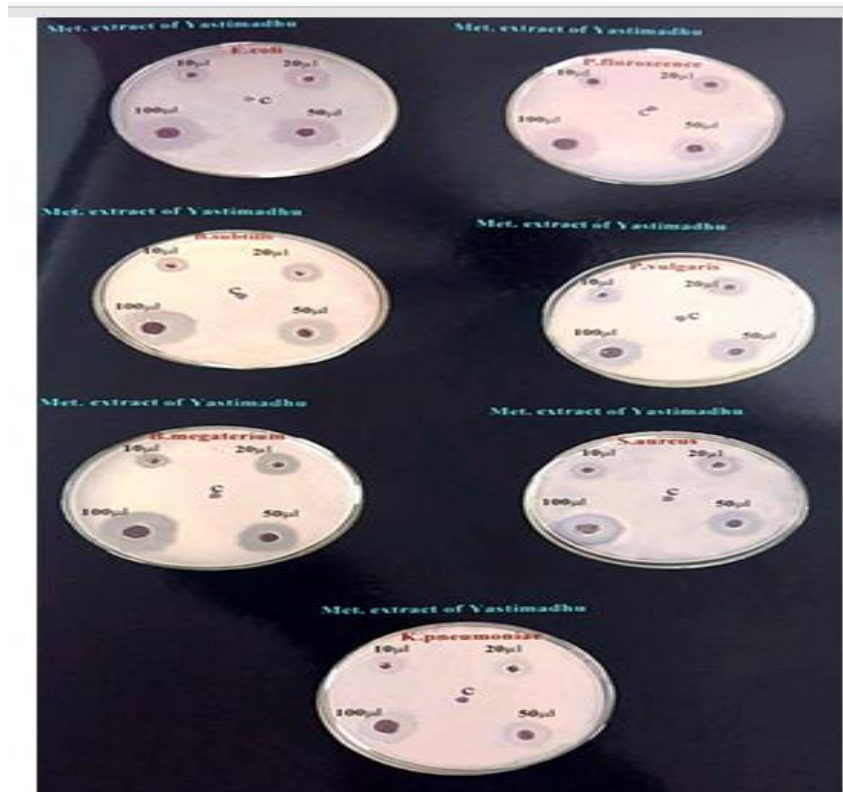


Figure 5: Antibacterial Activity of Methanolic extract of *Glycyrriza glabra* on Test Organisms

Organism	Type of Extract	Concentration of Extract (in $\mu$ l)			
		10	20	50	100
		Zone of Inhibition (in mm)			
<i>Escherichia coli</i>	Ethanol	-	-	-	-
	Methanol	4	6	8	10
<i>Proteus vulgaris</i>	Ethanol	-	-	-	-
	Methanol	3	4	6	9
<i>Staphylococcus aureus</i>	Ethanol	-	-	-	-
	Methanol	3	4	7	9
<i>Klebsiella pneumoniae</i>	Ethanol	-	-	-	-
	Methanol	3	4	5	6
<i>Pseudomonas fluorescense</i>	Ethanol	-	-	-	-
	Methanol	2	3	5	7
<i>Bacillus subtilis</i>	Ethanol	-	-	-	-
	Methanol	2	3	5	7
<i>Bacillus megaterium</i>	Ethanol	-	-	-	-
	Methanol	2	4	6	9

Table 6: Antibacterial Activity of Extract of *Glycyrrhiza glabra* on Test Organisms

**Graph 2.1: Antibacterial Activity of Ethanolic Extract of *Tinospora cordifolia* on Test Organisms**

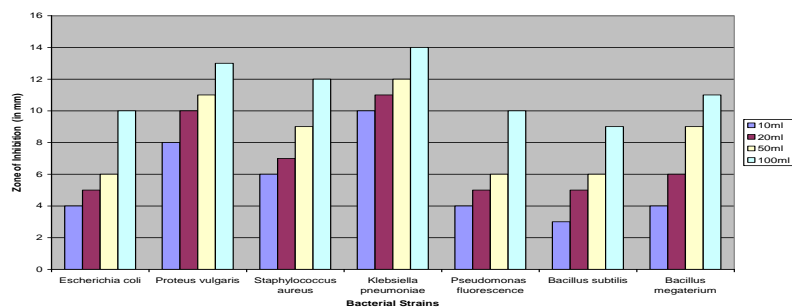


Figure 6: Anti-bacterial activity of ethanol extract of *Tinospora cordifolia* on test organisms

**Graph 2.2: Antibacterial Activity of Ethanolic Extract of *Rubia cordifolia* on Test Organisms**

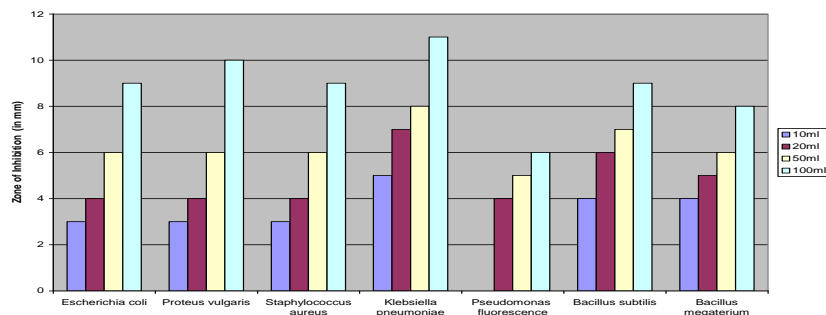


Figure 7: Anti-bacterial activity of ethanol extract of *Rubia cordifolia* on test organisms

Graph 2.3: Antibacterial Activity of Ethanolic and Methanolic Extract of *Saussurea lappa* on Test Organisms

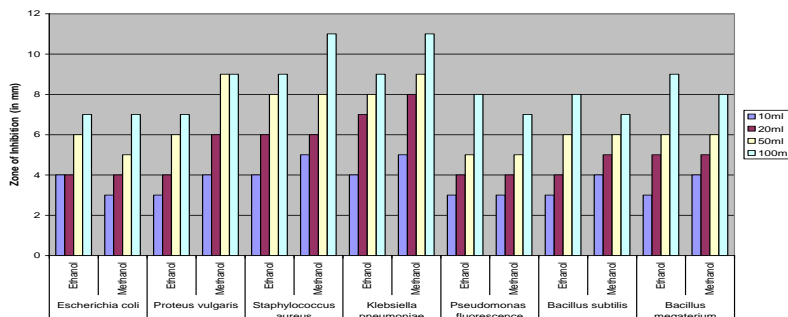


Figure 8: Anti-bacterial activity of ethanol and methanolic extract of *Saussurea lappa* on test organisms

Graph 2.4: Antibacterial Activity of Methanolic Extract of *Glycyrrhiza glabra* on Test Organisms

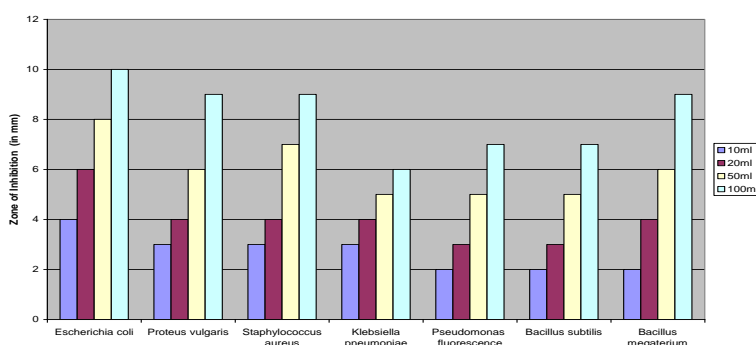


Figure 9: Anti-bacterial activity of methanolic extract of *Glycyrrhiza glabra* on test organisms

In this assay the extracts were prepared by using solvents like Methanol and Ethanol. The extracts showed increased solubility in these solvents and showed consistent antimicrobial activity. The antimicrobial assay was carried out on three Gram positive bacteria i.e., *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus* and four Gram Negative bacteria i.e., *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*.

#### Antimicrobial activity of ethanolic and methanolic extracts

The ethanolic extract of *Tinospora cordifolia* showed maximum antimicrobial activity against *Klebsiella pneumoniae* (14 mm), followed by *Proteus vulgaris* (13 mm), *Staphylococcus aureus* (12 mm), *Bacillus megaterium* (11 mm), *E. coli* and *Pseudomonas fluorescens* (10 mm), and *Bacillus subtilis* (9 mm) (Table 3). The collective analysis of antimicrobial activity of ethanolic extract indicated that among the four medicinal plants used in the study, *Tinospora cordifolia*, *Rubia cordifolia* have better impact ranged from 14 to 9 mm on the bacteria when compared *Saussurea lappa*, ranged from 9- 7mm whereas ethanolic extract of *Glycyrrhiza glabra* does not show any effect on bacteria (Table-6).

#### Antimicrobial activity of methanolic extract

The antimicrobial activity of methanolic extract of *Saussurea lappa* showed maximum zone of inhibition (11 mm) against *Staphylococcus aureus*, *Klebsiella pneumoniae*, followed by *Proteus vulgaris* (9 mm), *Bacillus megaterium* (8 mm) and *E. coli*, *Pseudomonas fluorescens*, *Bacillus subtilis* (7 mm) (Table 5). Similarly, the methanolic extracts of *Glycyrrhiza glabra* showed antimicrobial activity on *E. coli* (10 mm), *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus megaterium* (9 mm) and *Pseudomonas fluorescens*, *Bacillus subtilis* (7 mm), *Klebsiella pneumoniae* (6 mm) respectively (Table-6). Whereas the methanolic extract of *Tinospora cordifolia*, *Rubia cordifolia* does not showed any effect on the bacteria (Table- 3 & 4).

**Gram negative:** *E. coli* was found to be susceptible to Methanolic and Ethanolic extracts of *Saussurea* and Ethanolic extract of *Tinospora*. *Proteus vulgaris* was susceptible to *Tinospora* (ethanolic) and *Saussurea* (ethanolic and methanolic) extracts. *Klebsiella pneumoniae* was found to be susceptible to the Ethanolic extracts of *Tinospora* and Methanolic extracts of *Saussurea*.

**Gram positive:** *Pseudomonas aeruginosa* growth was inhibited by Methanolic and Ethanolic extracts of *Tinospora* and *Saussurea*. *Bacillus subtilis* and *Bacillus megaterium* were found susceptible to Methanolic and Ethanolic extracts of *Saussurea* and Ethanolic extract of *Tinospora*. *Staphylococcus aureus* was found susceptible to methanolic extract of *Saussurea* and Ethanolic extract of *Tinospora* and *Saussurea*.

**IV. Conclusion**

Owing to the medicinal importance of Ayurvedic plants the present work concentrated on studying the antimicrobial properties of some herbal plants available in Tirumala Hills and Tirupati of Andhra Pradesh. Plant extracts with known antimicrobial properties hold significant potential for therapeutic applications.

In the present study, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Escherichia coli* showed higher sensitivity and *Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus* showed moderate sensitivity and less sensitivity was observed in *Bacillus megaterium*.

Results obtained from this study revealed that, the plant extracts showed antimicrobial activity and they may use without any side effects whereas commercially available drugs sometimes show negative results on host.

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