Antimicrobial Efficacy of some Medicinal Plants Against Selected Bacterial Strains

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***Abstract*—This study explores the antimicrobial potential of four medicinal plants, in response to the increasing threat of antibiotic resistance in human pathogens. Methanol and ethanol and aqueous extracts of these plants were tested against seven bacteria, including *Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescence* and *Proteus vulgaris*. Using the agar well diffusion method, the extracts antimicrobial activity was evaluated by measuring the zone of inhibition. Results showed that the methanolic extracts demonstrated greater antibacterial activity compared to the ethanolic extracts. Aqueous extract of *Alium sativum* showed antimicrobial activity. These findings suggest that plant extracts with antimicrobial properties as therapeutic agents, offering a potential solution against antibiotic-resistant bacteria.**

***Index Terms*—Antimicrobial, Bacterial culture, ethanol, methanol and aqueous extracts, Medicinal plants,**

I. INTRODUCTION

The Tirumala and Tirupati hills, situated in the Chittoor District of Andhra Pradesh, are renowned for their rich biodiversity and cultural significance as the sacred abode of Lord Venkateshwara. Research has revealed that these hills harbor a diverse array of ethno-medicinal plants with immense potential for disease management. With the growing concern of antibiotic resistance, scientists are increasingly turning to medicinal plants as a source of new antimicrobial agents. Screening plant families for antibacterial properties is essential for discovering novel therapeutic agents. The phytochemical and health benefits of many medicinal plants remain unexplored, offering promising opportunities for future research [1, 2]. Traditional medicinal plants are often preferred in developing regions due to their affordability and limited access to synthetic alternatives [3].

Medicinal plants used in the present study

Piper longum (Pippali)

Pippali is one of the key medicinal plants used in Ayurveda, known for its ability to promote overall health and well-being. The plant is a climber, bearing black-colored fruits with a distinctive consistency. It typically grows in habitats near water streams [4].

Phytochemically, Pippali is rich in bioactive compounds such as piperine, piper longumine, and various lignans, which contribute to its anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, neuroprotective, and bio enhancing properties. Both preclinical and clinical studies support the potential of Pippali in managing a wide range of health conditions, including digestive disorders, metabolic syndrome, osteoporosis, neurodegenerative diseases, and as an adjunctive therapy in infectious diseases such as COVID-19 [5].

Phyllanthus emblica (Amla)

Phyllanthus emblica, commonly known as Indian gooseberry or Amla, contains a diverse range of secondary metabolites such as flavonoids, tannins, polyphenols, and ascorbic acid. These bioactive compounds are primarily responsible for its potent antioxidant, anti-inflammatory, and antimicrobial properties, highlighting its potential as a valuable source for natural therapeutics and pharmaceutical applications. Phyllanthus emblica demonstrates a broad range of pharmacological activities, including hepatoprotective and immunomodulatory effects, as well as significant antidiabetic, anticancer, and cardioprotective potentials.

In addition to vitamin C, Phyllanthus emblica fruit contains several other essential vitamins such as carotene, niacin, riboflavin, and thiamine. The fruit is notably rich in vitamin C (70%–72%) and comprises

various bioactive compounds, including tannins, phembembaic acid, gallic acid, lipids, emblicol, flavonoids, and mymic acid. The leaves of P. emblica are also abundant in phytochemicals such as gallic acid, chebulic acid, ellagic acid, kaempferol, kaempferol-3-O-glucoside, gallotannin, and rutin, along with phosphoric acid, essential oils, and fatty acids like linoleic, oleic, stearic, palmitic, and myristic acids [6].

Terminalia chebula (Haritaki)

Terminalia chebula, a drupe with a hard-coated seed, is commonly found in southern India, where its fruits (myrobalans) are traditionally used in complementary medicine. In Ayurveda and Siddha systems, these fruits are valued for their therapeutic potential in treating various ailments, including digestive disorders, respiratory conditions, skin diseases, and cardiovascular problems. Additionally, they are employed to alleviate symptoms of diabetes, epilepsy, and memory loss, and are known for their homeostatic, diuretic, antitussive, and wound-healing properties [7, 8].

Alium sativum (Lasuna)

Alium sativum L. (garlic) is a widely utilized medicinal and culinary plant belonging to the family Amaryllidaceae. It is recognized globally for its diverse pharmacological and therapeutic properties. The bulb of *A. sativum* contains a rich array of bioactive sulfur-containing compounds, such as allicin, alliin, and ajoene, which contribute to its characteristic aroma and potent biological activities [9]. Numerous studies have demonstrated that these constituents exhibit immunomodulatory and immunostimulatory effects, thereby enhancing host defense mechanisms. Traditionally, *A. sativum* has been employed in the management of cardiovascular disorders, neoplastic conditions, rheumatism, diabetes mellitus, helminthic infections, gastrointestinal disturbances, hepatic dysfunctions, and respiratory ailments including bronchitis and tuberculosis. It has also been reported to exert antihypertensive, hepatoprotective, antimicrobial, and antioxidant effects, supporting its broad therapeutic potential in ethnomedicine [10]. In India, A. sativum is used to treat fever, coughs and is administered topically against scabies, graying of hair, and eczema, as well as against inflammation of the tetanus and lungs.

S. No	Plant Name	Common Name in Telugu	English Name	Sanskrit Name	Hindi Name	Parts used	Family	Medicinal Properties
1	*Piper longum*	Pippallu	Dried catkins	Pippali, Magadhi, ushana, kana	Pipar, Piplamul	Fruit	Piperaceae	Cure respiratory tract diseases, cough, bronchitis, asthma
2	*Phyllanthus emblica*	Usiri, Nelli, Usirikayi, Triphalamu, Peddausiri	Indian gooseberry	Phyllanthus Amala, amalaka, dhatriphala	Amla, Amalaki	Fruit	Euphorbiaceae	Antibacterial, anti-viral, antipyretic, antioxidative
3	*Terminalia chebula*	Karakkaya	Inkunt, Chebulic Myrobalo	Haritaki, Abhaya, Pathya	Harad, Haritali	Fruit	Combretaceae	Helps in removing toxins from the body
4	*Aliumsativum*	Vellulli	Garlic	Lasuna	Lasan,Lasun	Bulbs	Alliaceae	anorexia, constipation, antiseptic, antimalarial, anti-filarial, antispasmodic in asthma, antihelminthic

Table-1: Details and medicinal values of the plants used in the present study

Fig-1: Plants and their parts used in the present 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 fluorescence, 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
Staphylococcus aureus	Gram positive	Cocci
Bacillus subtilis		Rod
Bacillus megaterium		Rod
Escherichia coli	Gram negative	Rod
Klebsiella pneumoniae		Rod
Pseudomonas fluorescence		Rod
Proteus vulgaris		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 4ºC. 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., *Piper longum, Phyllanthus emblica, Terminalia chebula, Alium sativum* 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 fluorescence* and *Proteus vulgaris.* All the medicinal plant extracts used against the bacteria have showed diverse degree of antimicrobial activity against the pathogens.

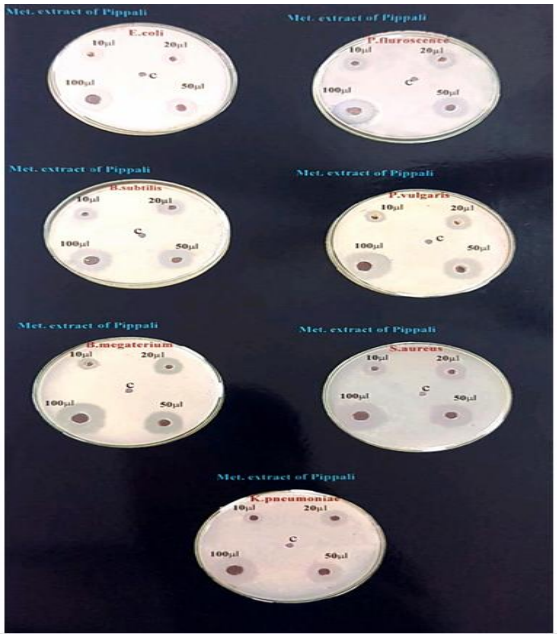

Figure:2 Methanolic extract of *Piper longum* on test organisms

Organism	Type of Extract	Concentration of Extract (in µl)			
		10	20	50	100
		Zone of Inhibition (in mm)			
Escherichia coli	Ethanol	-	-	-	-
	Methanol	3	4	5	6
Proteus vulgaris	Ethanol	-	-	-	-
	Methanol	3	4	6	10
Staphylococcus aureus	Ethanol	-	-	-	-
	Methanol	4	6	8	11
Klebsiella pneumoniae	Ethanol	-	-	-	-
	Methanol	3	5	7	9
Pseudomonas fluorescence	Ethanol	-	-	-	-
	Methanol	3	4	6	9
Bacillus subtilis	Ethanol	-	-	-	-
	Methanol	3	4	6	7
Bacillus megaterium	Ethanol	-	-	-	-
	Methanol	3	5	7	10

Table 3: Antibacterial Activity of methanolic extract of *Piper longum* on Test Organisms

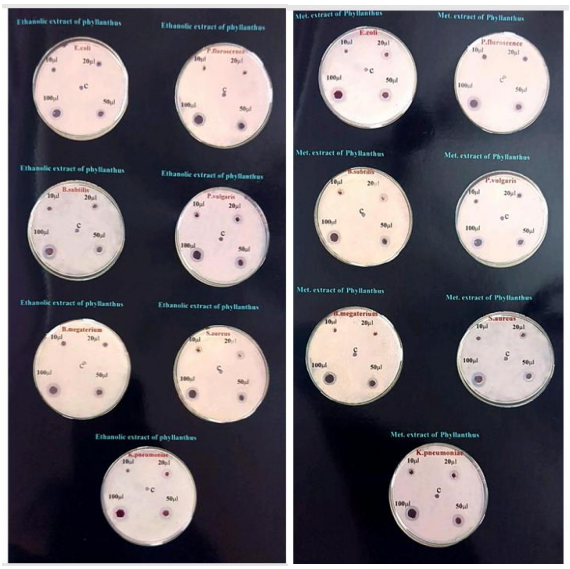

Figure 3: Ethanolic and Methanolic extract of Phyllanthus emblica

Table 4: Antibacterial Activity of Ethanolic and methanolic extract of *Phyllanthus emblica* on Test Organisms

Organism	Type of Extract	Concentration of Extract (in µl)			
		10	20	50	100
		Zone of Inhibition (in mm)			
Escherichia coli	Ethanol	-	2	3	4
	Methanol	-	2	4	6
Proteus vulgaris	Ethanol	-	2	4	6
	Methanol	-	2	3	4
Staphylococcus aureus	Ethanol	2	3	4	5
	Methanol	-	2	3	5
Klebsiella pneumoniae	Ethanol	-	3	5	6
	Methanol	-	2	4	5
Pseudomonas fluorescence	Ethanol	-	-	3	4
	Methanol	-	-	2	4
Bacillus subtilis	Ethanol	-	2	3	4
	Methanol	2	3	4	5
Bacillus megaterium	Ethanol	-	-	3	5
	Methanol	-	-	3	4

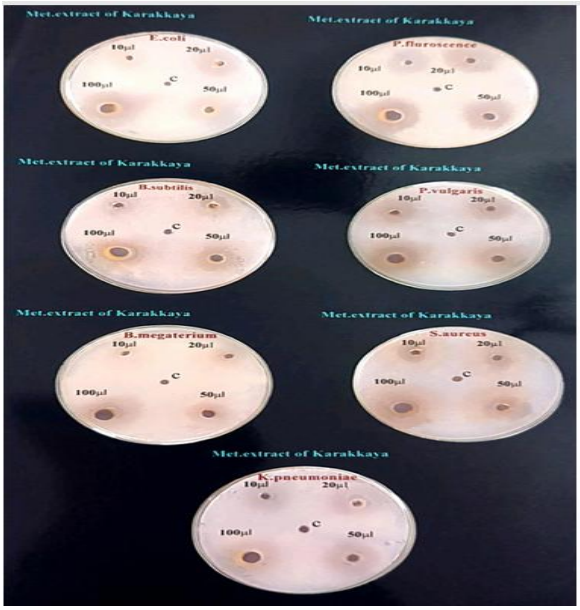

Figure-4: Ethanolic and methanolic extract of *Terminalia chebula*

Organism	Type of Extract	Concentration of Extract (in µl)			
		10	20	50	100
		Zone of Inhibition (in mm)			
Escherichia coli	Ethanol	-	3	5	9
	Methanol	7	9	11	13
Proteus vulgaris	Ethanol	4	6	7	9
	Methanol	8	9	10	11
Staphylococcus aureus	Ethanol	3	5	6	7
	Methanol	5	6	9	10
Klebsiella pneumoniae	Ethanol	5	7	8	9
	Methanol	7	10	11	12
Pseudomonas fluorescence	Ethanol	4	4	5	9
	Methanol	8	9	11	13
Bacillus subtilis	Ethanol	5	7	9	10
	Methanol	4	5	6	10
Bacillus megaterium	Ethanol	4	6	7	9
	Methanol	-	2	6	10

Table-5: Antibacterial Activity of ethanolic and methanolic extract of *Terminalia chebula* on Test Organisms

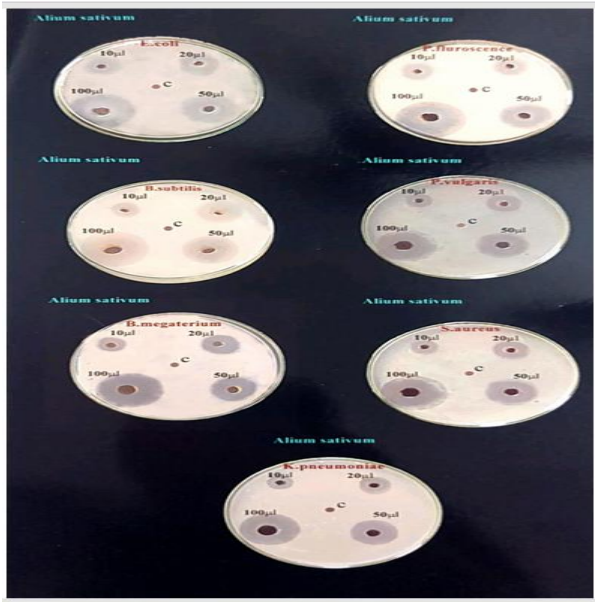

Figure- 5: Aqueous extract of *Alium sativum*

Organism	Type of Extract	Concentration of Extract (in µl)			
		10	20	50	100
		Zone of Inhibition (in mm)			
Escherichia coli	Aqueous	6	8	11	13
	Ethanol	-	-	-	-
	Methanol	-	-	-	-
Proteus vulgaris	Aqueous	3	4	8	11
	Ethanol	-	-	-	-
	Methanol	-	-	-	-
Staphylococcus aureus	Aqueous	3	4	7	11
	Ethanol	-	-	-	-
	Methanol	-	-	-	-
Klebsiella pneumoniae	Aqueous	3	5	6	9
	Ethanol	-	-	-	-
	Methanol	-	-	-	-
Pseudomonas fluorescence	Aqueous	5	6	11	13
	Ethanol	-	-	-	-
	Methanol	-	-	-	-
Bacillus subtilis	Aqueous	4	6	9	11
	Ethanol	-	-	-	-
	Methanol	-	-	-	-
Bacillus megaterium	Aqueous	4	5	7	11
	Ethanol	-	-	-	-
	Methanol	-	-	-	-

Table-6: Antibacterial Activity of aqueous extract of *Alium sativum* on Test Organisms

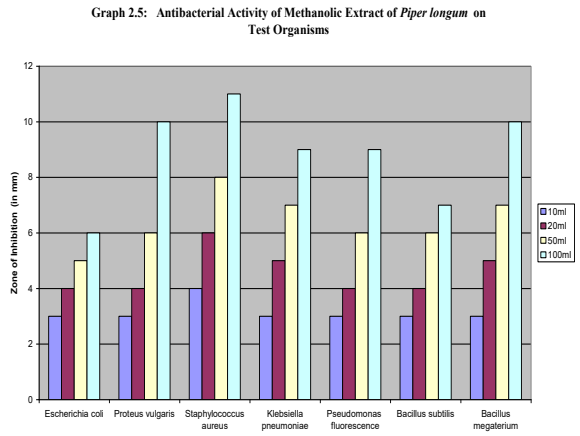

Figure 6: Anti-bacterial activity of ethanol extract of Piper longum on test organisms

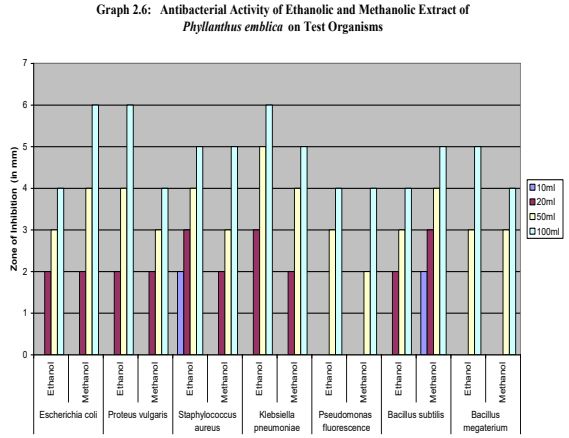

Figure 7: Anti-bacterial activity of ethanolic and methanolic extract of Phyllanthus emblica on test organisms

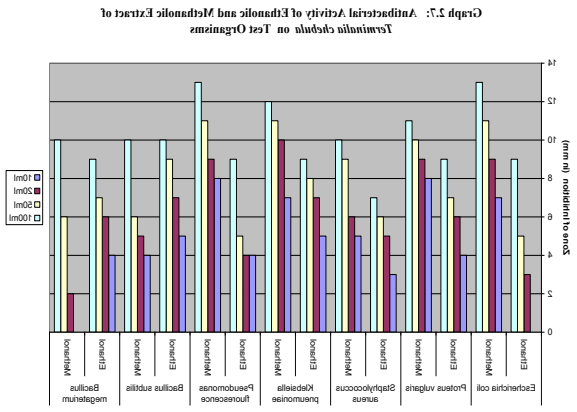

Figure 8: Anti-bacterial activity of ethanolic and Methanolic extract of Terminalia chebula on test organisms

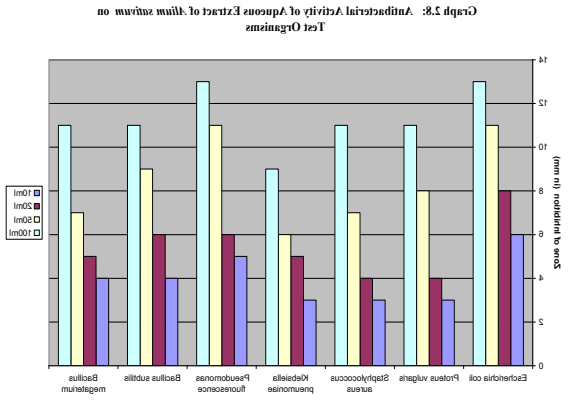

Figure 9: Anti-bacterial activity of aqueous extract of Alium sativum on test organisms

IV. DISCUSSION OF FINDINGS

In this assay the extracts were prepared by using solvents like Methanol and Ethanol and aqueous extract for Alium sativum. 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

Antimicrobial activity of ethanolic extract

The ethanolic extract of *Piper longum and Alium sativum* does not show any activity on the organisms (Table 3 & 6) whereas *Phyllanthus emblica* showed maximum antimicrobial activity against Klebsiella pneumoniae and Proteus vulgaris (6 mm), followed by Staphylococcus aureus, Bacillus megaterium (5 mm), E. coli, Pseudomonas fluorescence and Bacillus subtilis (4 mm) (Table- 4). *Terminalia chebula* showed maximum antimicrobial activity against Bacillus subtilis (10 mm), followed by E. coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas fluorescence and Bacillus megaterium (9 mm), and Staphylococcus aureus (7 mm) (Table- 5). The collective analysis of antimicrobial activity of ethanolic extract indicated that among the four medicinal plants used in the study, *Terminalia chebula,* have better impact ranged from 10 to 7 mm on the bacteria (Table- 5), when compared

Phyllanthus emblica, ranged from 6- 4 mm (Table- 4), whereas *Piper longum and Alium sativum,* does not show any effect on bacteria (Table 3 & 6).

Antimicrobial activity of methanolic extract

The antimicrobial activity of methanolic extract of *Terminalia chebula* showed maximum zone of inhibition (13 mm) against E. coli, Pseudomonas fluorescence followed by Klebsiella pneumoniae (12mm), Proteus vulgaris (11 mm), Staphylococcus aureus, Bacillus megaterium and, Bacillus subtilis (10 mm) (Table 5). Similarly, the methanolic extracts of *Piper longum* showed antimicrobial activity on Staphylococcus aureus (11mm) followed by Proteus vulgaris, Bacillus megaterium (10 mm), Klebsiella pneumoniae, Pseudomonas fluorescence (9mm) Bacillus subtilis (7 mm), and E. coli (6 mm) respectively (Table-3). *Phyllanthus emblica* methanolic extracts showed antimicrobial activity on E. coli (6 mm), followed by Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis (5 mm). Proteus vulgaris, Pseudomonas fluorescence, Bacillus megaterium (4 mm) (Table- 5). Whereas the methanolic extract of *Alium sativum* does not showed any effect on the bacteria (Table- 6).

Antimicrobial activity of Aqueous extract of Alium sativum

The ethanolic and methanolic extracts of *Alium sativum* did not show any effect on the bacteria (Table-6). But the aqueous extract showed maximum zone of inhibition (13 mm) against *E. coli, Pseudomonas fluorescence* followed by *Staphylococcus aureus, Proteus vulgaris, Bacillus megaterium* and*, Bacillus subtilis* (11 mm) *Klebsiella pneumoniae* (9mm) (Table 6).

Gram negative: E. coli, Proteus vulgaris and Klebsiella pneumoniae were found to be susceptible to Methanolic and Ethanolic extracts of Phyllanthus and Terminalia. Methanolic extract of Piper and Aqueous extract of Alium.

Gram positive: Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and Bacillus megaterium growth was inhibited by Methanolic and Ethanolic extracts of Phyllanthus and Terminalia. Methanolic extract of Piper and Aqueous extract of Alium.

V. 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 this assay the extracts were prepared using sterile distilled water and solvents like Methanol and Ethanol. We found the Ethanolic and Methanolic plant extracts showed consistent antimicrobial activity. This might have resulted from the lack of solubility of active constituents in aqueous solutions while the extracts showed increased solubility in solvents like Methanol and Ethanol, except in *Alium sativum* which showed higher antimicrobial activity in aqueous extract.

In the present study, the Methanolic extract of *Terminalia* showed higher antimicrobial activity, later *Piper longum* and *Phyllanthus.* In the same way ethanolic extract of *Terminalia* showed higher antimicrobial activity than *Phyllanthus*. The Aqueous extract of *Alium sativum* showed higher activity and ethanolic and methanolic extracts did not showed any impact on the selected bacterial strains.

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 the host.

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