

Antimicrobial activities of selected medicinal plants against bacteria and fungi

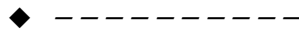
B. Annapoorna, R. Ranjani

Department of Virology

Sri Venkateswara University, Tirupati-517502, A.P., India

ABSTRACT: Microbial organisms such as virus, bacteria and fungi can survive in all environmental conditions in the world and they can withstand high temperature, pressure, and pH. They infect plants, animals and human beings, they cause severe damage to host and lead to lot of economic loss. Medicinal plants are used widely for treating various diseases caused by protozoans, helminthes, bacteria, fungus and viruses. Many drugs are identified from medicinal plants and are active against microbes. But still there is a need for identification of new efficient drugs. Most of the drugs are available for several diseases but usage of natural drugs is more efficient, safe and cheap when compare with the synthetic drugs. Therefore author wants to focus on antimicrobial activity, including antibacterial and antifungal activity of medicinal plants and herbs. Five medicinal plants with five different solvents were used to study antibacterial and antifungal activities.

Index Terms— antimicrobial, antibacterial, antifungal, medicinal plants, bacteria, fungi, plant drug -----



1 INTRODUCTION

Microbial organisms such as virus, bacteria and fungi cause disease and lead to decrease the quality of human life. Identification of effective and natural drug can help in protecting from diseases. Medicinal plants are the natural resources which are used to treat several diseases caused by bacteria, fungi and viruses. Because medicinal plants have secondary metabolites such as alkaloids, carbohydrates, flavonoids, glycosides, sterols, saponins, terpenoids, coumarins, quinones and tannins (Cowan, 1999). Only one third of the infectious diseases have been treated by these synthetic products (Manish Kumar et.al., 2014, Sharma 2011) Usage of natural drugs are more efficient then chemical drugs and no side effects. There is an increasing need to search for new compounds with antibacterial and antifungal activity to microbial infections. The available commercial (Field and Biron 1994), antimicrobial drugs are often unsatisfactory due to the problem of microbial resistance in various strategies of their life cycle (Hancock, et.al., 2012), the problem of viral latency and conflicting efficacy in recurrent infection in immune compromised persons. Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial 2.

MATERIAL AND METHODS

2.1 Collection of plant

Medicinal plants are collected during the growing season of January to May from different places of Bonupalli, surroundings of Tirupathi and Tirumala hills in Chittoor district, Andhra Pradesh. Collected plants are identified with plant taxonomist, Dept. of Botany, S V Arts College, Tirupathi, Andhra Pradesh, India. Among all, the five important plants were identified and selected for further studies. And they are Boer-

haavia diffusa, Eclipta alba, Sphaeranthus indicus, Dodonaea viscosa, Melia azadirachL (leaf and bark).

2.2 Preparation of plant extract

Preparation of extractions according to the method of Alam Sher (2009). The collected five different plant materials was shade dried and prepared powder mechanically using an electrical stainless steel blender. Different solvents such as water, Hexane, Ethyl acetate, Methanol, Methanol plus Water were used to prepare extracts with selected five plant material.

The plant extract were prepared according to the method of Alam Sher (2009). The selected different five plant material was air dried and powdered. The prepared powder was taken in to 50 ml of different solvent such as water, Hexane, Ethyl acetate, Methanol, Methanol plus Water in separate container and prepared extracts. And the plant extract were used for experimental purpose.

2.3 Test microorganisms

Microbial pathogens: Bacterial and species like: Streptococcus pneumonia = S. pneumonia; Staphylococcus aureus = S. aureus; Pseudomonas aeruginosa = P.aeruginosa; Bacillus subtilis = B. subtilis; Exteuricia coli = E.Coli; Lacto bacillus = L.Bacillus;

Fungal species like Aspergillus niger = A,niger; Aspergillus flavous = A.flavous; Trichoderma harzianum = T.harzianum; Pencillium notatum= P.notatum; Alternaria arborescens= A. arborescens were used to study antimicrobial activities.

2.4 Activity test

2.4.1 Antibacterial activity

Antibacterial activities are tested nutrient agar well diffusion method by S. Joshi et al., (2008), Murray et al., (1995) later modified by Olurinola (1996). According to the method, nutrient agar (40gr/liter) were taken for culturing of bacteria. Minimum Bacterial concentrations (MBC) and Minimum inhibitory Concentrations (MIC) are determined. The different concentrations of plant samples are added in to the wells then the plates were incubated in incubator for 20 -24hr for antibacterial activity. Final zone of inhibition is calculated by subtracting control zone with test sample zone of inhibition. Resultant zone is taken as final as zone of inhibition of test sample.

2.5 Antifungal activity

Antifungal activities are tested by agar well diffusion method (Joshi et al., 2008, Murray et al., 1995), later modified by Olurinola (1996) According to the method, Czapadox agar medium (39gr/liter) were taken for culturing of fungi. Minimum Fungal Concentrations (MFC) and Minimum inhibitory Concentrations (MIC) are determined. The different concentrations of plant samples are added in to the wells then the plates were incubated in incubator for 36 to 48 hr for antifungal activity. Final zone of inhibition is calculated by subtracting control zone with test sample zone of inhibition. Resultant zone is taken as final as zone of inhibition of test sample.

3. RESULT AND DISCUSSION

In the present study of investigation, different solvent extracts are used for the inhibitory activity against both fungi and bacteria by well diffusion method under in vitro conditions, and zone of inhibition is calculated by subtracting control zone from the test sample zone and resultant zone is taken as actual zone of inhibition.

Plant extracts of *Eclipta alba*, *Boerhaavia diffusa*, *Melia azedarach* L. *Melia azedarach* Linn leaf, *Sphaeranthus indicus*, *Dodonaea viscosa* and *Melia azedarach* L. bark were evaluated by measuring the zone of inhibition against the pathogens were compared with the control and test sample. Among the different solvent extracts were examined, the methanol extract has showing the high degree of effect when compared to the other solvents then methanol plus water extract (50% methanol), Ethyl acetate, water and then hexane. For the entire microbes methanol and 50% methanol extracts of plant *Sphaeranthus indicus* and *Boerhaavia diffusa* has showing maximum activity zone of inhibition 0.9 cm and 0.2 cm of diameter respectively for *Staphylococcus aureus* with ethyl acetate extract, 1.1 cm and 1.3 cm of diameter inhibition zone respectively occurs for *Pseudomonas*, and 1.0 cm and 0.7 cm of diameter zone of inhibition occurs for *Lacto bacillus* with ethyl acetate extract. Methanol extract of above plant extracts showing the 0.2 cm and 1.0 cm of diameter of inhibition zone for *Streptococcus pneumoniae*, 1.0 cm and 0.3 cm of diameter of zone of inhibition was obtained for *Bacillus* species, 0.2 cm and 0.6 cm of diameter zone of inhibition occurs for *E. coli*, and 0.8 cm zone of inhibition zone for *Streptococcus pneumoniae*, 0.6 cm of

diameter for *Pseudomonas* and 1.0 cm of diameter of zone occurs for *L. bacillus* with methanol extract of *Boerhaavia diffusa*.

The antifungal activity of *Boerhaavia diffusa* and *Sphaeranthus indicus* are showing the maximum activity. In that *Boerhaavia diffusa* exhibiting the activity in all the solvents including water extract, but maximum zone of diameter occurs with methanol extract, ethyl acetate then 50% methanol, hexane then aqueous extract. The maximum zone of diameter for that is 1.1 cm of diameter zone of inhibition for *A. niger*, 1.0 cm of diameter zone of inhibition for *A. flavus*, 0.7 cm of diameter zone of inhibition for *Alternaria arborescens*, 0.6 cm of diameter zone of inhibition for *Penicillium notatum* and 0.8 cm of diameter zone of inhibition for *Trichoderma aggressivum*.

Graphical representations of six different plants with five different solvents are both bacterial and fungal examines are as follows by specifying with single plant compound with different solvent extracts on different Bacterial and fungal cultures.

3.1 Antibacterial and antifungal activity of *Sphaeranthus indicus*

Ethyl acetate extract of *S. indicus* has antibacterial activity against *Pseudomonas* (1.7 - 0.6 = 1.1 cm), *L. bacillus* (1.4 - 0.6 = 0.8 cm), and *Staphylococcus* (1.5 - 0.6 = 0.6 cm). Methanol extract of *S. indicus* extract has antibacterial activity against *E. coli* (1.2 - 0.6 = 0.6 cm) *Bacillus* (1.0 - 0.6 = 0.4 cm) *Streptococcus* (0.7 - 0.6 = 0.1 cm). 50% Methanol extract of *Sphaeranthus* has Inhibitory property on *Staphylococcus* (1.4 - 0.6 = 0.8 cm) and *Streptococcus* (0.8 - 0.6 = 0.2 cm). *S. indicus* water extract shows inhibitory activity shows on *A. flavus* (0.7-0.6 = 0.1cm). *S. indicus* hexane extract shows inhibitory activity shows on *A. flavus* (0.8-0.6=0.2cm), *Penicillium* (0.8-0.6 = 0.2cm) *Trichoderma* (0.8-0.6 = 0.2cm) *Alternaria* (0.7-0.6 = 0.1cm) and *A. flavus* (0.7-0.6 = 0.1cm). *S. indicus* ethyl acetate extract shows inhibitory activity shows on *A. niger* (0.7-0.6 = 0.1cm). *S. indicus* methanol extract shows inhibitory activity shows on *Alternaria* (1.1-0.6 = 0.5cm). *S. indicus* methanol extract shows inhibitory activity shows on *A. flavus* (0.7-0.6 = 0.1cm). *E. alba* 50% methanol extract was active against *Penicillium* (0.8cm-0.6cm = 0.2cm) (Fig. 3.1).

Ethyl acetate extract of *D. viscosa* has antibacterial activity against *L. bacillus* (1.4 - 0.6=0.8 cm), *D. viscosa* methanol extract has antibacterial activity against *Pseudomonas* (1.2 - 0.6 =0.6 cm), *Staphylococcus* and *E. coli* (1.0 - 0.6 = 0.4 cm), 50% methanol extract of *D. viscosa* extract shows inhibitory effect on *Staphylococcus* (0.8 - 0.6 = 0.2 cm).

Water extract *D. viscosa* shows antifungal activity on *A. flavus* (0.7-0.6 = 0.1cm). *D. viscosa* hexane extract shows antifungal activity on *Alternaria* (0.8-0.6 = 0.2cm) *Trichoderma* (0.7-0.6 = 0.1cm) *A. niger* (0.7-0.6 = 0.1cm). *D. viscosa* ethyl acetate extract shows antifungal activity on *A. niger* (0.9-0.6 = 0.3cm). *Penicillium* (0.8-0.6 =0.2cm) *Trichoderma* (0.7-0.6 = 0.1cm). *D. viscosa* methanol extract shows antifungal activity on *A. niger* (1.1-0.6 = 0.5cm), *A. flavus* (1.0-0.6 = 0.4cm), *Alternaria* (0.9-0.6=0.3cm), *Trichoderma* (0.8-0.6 = 0.2cm). *D. viscosa* 50% methanol extract shows antifungal activity on *Trichoderma* (0.7-0.6 = 0.1cm) (Fig. 3.2).

3.3 Antibacterial and antifungal activity of *Boerhaavia diffusa*

Eclipta alba - water extract shows inhibitory activity

on *Bacillus* (1.2-0.6=0.6 cm). Hexane extract of *E. alba* exhibiting high inhibitory activity on *Staphylococcus* (2.5-0.6=1.9 cm), *E. alba* methanol extract has antibacterial activity against *Pseudomonas* (1.2 - 0.6 = 0.6 cm), *E. coli* (1.0 - 0.6 = 0.4 cm), *Bacillus* (0.9 - 0.6 = 0.3 cm), *Staphylococcus* and *Streptococcus* (0.8 - 0.6 = 0.2 cm). 50% Methanol extract of *E. alba* exhibiting high inhibitory activity on *Staphylococcus* (2.0 - 0.6 = 1.4 cm), and *Streptococcus* (1.6 - 0.6 = 1.0 cm). *E. alba* - hexane extract was active against *A.niger* (0.9cm-0.6cm = 0.3cm), *Alternaria* (0.8-0.6 = 0.2cm), *Trichoderma* (0.8-0.6 = 0.2cm) *Penicillium*(0.7-0.6 = 0.1cm) and *A.flavous* (0.7-0.6 = 0.1cm). *E. alba* ethyl acetate extract was active against *A. flavous* (1.2cm-0.6cm = 0.6cm), *A. niger* (1.0-0.6 = 0.4cm), *Alternaria* (0.8-0.6 = 0.2cm), *Trichoderma* (0.7-0.6 = 0.1cm). *E. alba* methanol extract was active against *Penicillium* (0.8cm-0.6cm = 0.2cm), *E. alba* 50% methanol extract was active against *Penicillium* (0.8cm-0.6cm = 0.2cm). (Fig. 3.4)

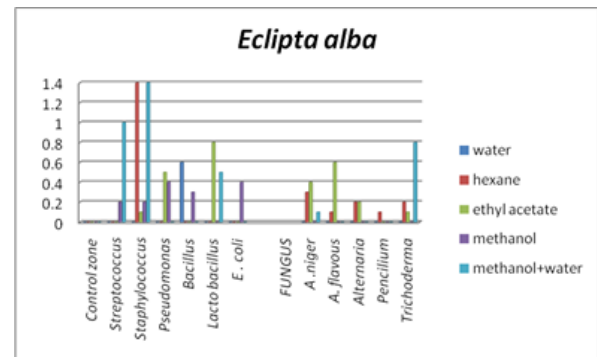


Figure: 3.4 Antibacterial and antifungal activity of *Eclipta alba*

3.5 Antibacterial and antifungal activity of *Melia azedarach* (Linn) leaf

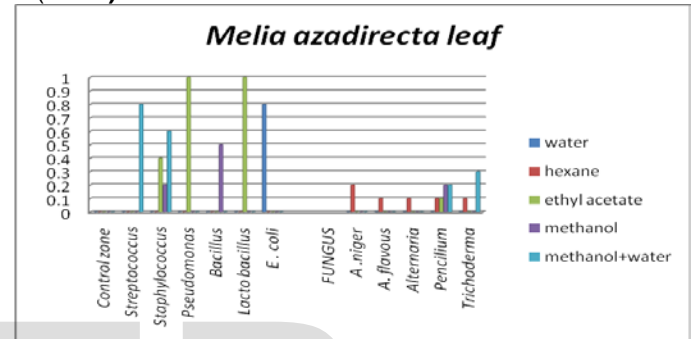


Figure: 3.5 Antibacterial and antifungal activity of *Melia azedarach* (Linn) leaf

Water extract of *Melia* leaf was active against *E.coli* (1.4-0.6=0.8 cm), *Melia* leaf hexane extract has antibacterial activity against *Staphylococcus* (2.0-0.6=1.4 cm). *Melia* leaf methanol extract has antibacterial activity against *Bacillus* (1.0 - 0.6 = 0.4 cm) *Streptococcus* (0.8 - 0.6 = 0.2 cm). *Melia* leaf 50%methanol extract has antibacterial activity against *Streptococcus* (1.4 - 0.6 = 0.8 cm) and *Staphylococcus* (1.2 - 0.6 = 0.6 cm). *Melia* leaf water was active against *A.niger* (0.8-0.6 = 0.2cm), and *A.niger* (0.7-0.6 = 0.1cm), *Alternaria* (0.7-0.6 = 0.1cm), *Penicillium* (0.7-0.6 = 0.1cm), *Trichoderma* (0.7-0.6 = 0.1cm). *Melia* leaf hexane extract shows antifungal activity on *Trichoderma* (0.9-0.6=0.3cm) *Alternaria* (0.8-0.6 = 0.2cm) *A. niger* (0.8-0.6 = 0.2cm). *Melia* leaf ethyl acetate extract shows antifungal activity on *Penicillium* (0.8-0.6 = 0.2cm). *Melia* leaf methanol extract shows antifungal activity on *Penicillium* (0.8-0.6 = 0.2cm). *Melia* leaf 50% methanol extract shows antifungal activity on *Trichoderma* (0.9-0.6 = 0.3cm) *Penicillium* (0.8-0.6 = 0.2cm). (Fig. 3.5)

3.6 Antibacterial and antifungal activity of *Melia azedarach* L. Bark

Melia bark water extract shows antibacterial activity on *Bacillus* (1.1-0.6=0.5 cm) and *Pseudomonas* (0.9-0.6=0.3 cm). *Melia* bark Ethyl acetate extract has antibacterial activity against *Pseudomonas* (1.2 - 0.6 = 0.6 cm) and *Staphylococcus* (0.8 - 0.6 = 0.2 cm). *Melia* bark methanol extract has antibacterial activity against *Staphylococcus* (1.8 - 0.6 = 1.2 cm), *Bacillus* (1.7 - 0.6 = 1.1 cm). *Pseudomonas* and *E. coli* (1.0 - 0.6 = 0.4 cm), *Streptococcus* (0.8 - 0.6 = 0.2 cm). *Melia* bark 50% methanol extract was active against *Staphylococcus* (1.4 - 0.6 = 0.8 cm) and *Streptococcus* (1.2 - 0.6 = 0.6 cm). *Melia* bark water extract

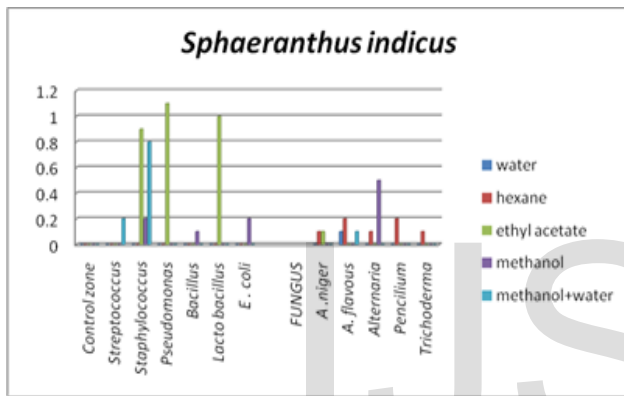


Figure: 3.1 Antibacterial and antifungal activity of *Sphaeranthus indicus*

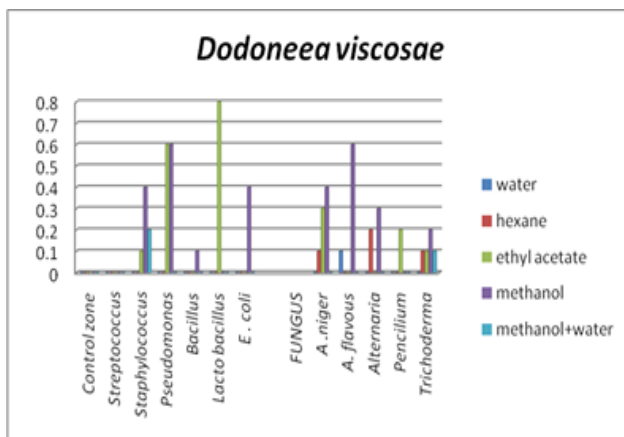


Figure: 3.2 Antibacterial and antifungal activity of *Dodonea viscosa*

shows antifungal activity on *A.flavous* (0.7-0.6 = 0.1cm). *Melia* bark was active against *Trichoderma* (0.9-0.6 = 0.3cm), *A.niger* (0.8-0.6 = 0.2cm), *Alternaria* (0.8-0.6 = 0.2cm). *Melia* bark ethyl acetate was active against *A.flavous* (1.1-0.6 = 0.5 cm), *A.niger* (0.8-0.6 = 0.2cm), *Alternaria* (0.7-0.6 = 0.1cm), *Trichoderma* (0.7-0.6 = 0.1cm). *Melia* bark 50% methanol extract shows antifungal activity on *A.niger* (1.1-0.6 = 0.5cm), *Trichoderma* (0.7-0.6 = 0.1cm) (Fig. 3.6).

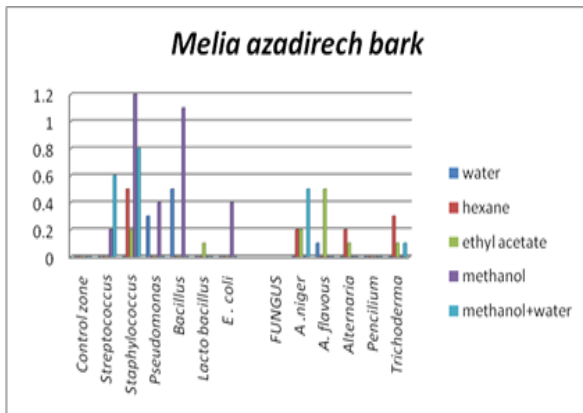


Figure: 3.6 Antibacterial and antifungal activity of *Melia azedarach* L. Bark

From thousands of years plant has therapeutic agents used as traditional medicine and cures several diseases. Medicinal plants play an important role in the health care by having the source of natural therapeutic agents with less toxic antibacterial agents, antifungal agents, antihelminthes agents (Venkatesan et al., 2009, Eloff 1998), and antiviral agents used in the production of novel drugs. Plants such as *Eclipta alba*, *Boerhaavia diffusa*, *Melia azedarach* L. leaf, *Sphaeranthus indicus*, *Dodonaea viscosa*, *Melia azedarach* L. bark were found inhibitory activity against all the bacteria and fungi we tested at 100ppm of concentration of sample. This broad spectrum activity due to the presence of novel secondary metabolites. Aqueous extract showing moderate activity and hexane extract showing minimum antimicrobial activity. Ethyl acetate extract showing the moderate activity, and methanol extract and 50%methanol extract showing the activity against the tested microbes.

4. CONCLUSION

In the present study of investigation, different extracts of above plants were evaluated for their antibacterial activity against certain bacteria, and fungus. Susceptibility of each plant extract was tested and determined by agar well diffusion method. Our preliminary investigation shows that all plant extracts of methanol, Ethyl acetate and water plus methanol mix are active against pathogenic microbial organisms. Finally we conclude that in the present investigation *Sphaeranthus indicus* has potential antibacterial activity and *Boerhaavia dif-*

fusa has potential antifungal activity. Further research can help to identify active antimicrobial compounds.

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