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.

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 Boerhaavia diffusa, Eclipta alba, Sphaeranthus indicus, Dodonaea viscosa, Melia azadirach. (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 azedarachL. Melia azedarach Linn leaf, Sphaeranthus indicus, Dodonaea viscosa and Melia azedarachL. 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 respective 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 Pencillium 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 = 0.9 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. flavous (0.7-0.6 = 0.1cm). S. indicus hexane extract shows inhibitory activity shows on A.flavous (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.flavous (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. flavous (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.flavous (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.flavous (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. flavus (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: 3.1 Antibacterial and antifungal activity of Sphaeranthus indicus

Figure: 3.2 Antibacterial and antifungal activity of Dodonaea viscosa

Figure: 3.4 Antibacterial and antifungal activity of Eclipta alba

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 ethyl acetate 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 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 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
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).

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

5. ACKNOWLEDGMENTS

The authors wish to thank Prof. D.V.R. Sai Gopal, Dept. of virology for providing facilities to complete this part of work.

REFERENCES


