

STUDIES ON IN VITRO ANTIBACTERIAL AND ANTIFUNGAL PROPERTY OF SPHAERANTHUS AMARANTHOIDES

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ABSTRACT

The control of microorganism is critical for the prevention and treatment of diseases. Plants are the rich source of antimicrobial agents. There is a tremendous increase in the prevalence of pathogenic microorganisms which are resistant to the existing antibiotics. So it is necessary to find out some new anti microbial agents from natural sources which are free from side effects. In the present study, various extracts of the plant *Sphaeranthus amaranthoides* were investigated for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Shigella boydii*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila*. For antifungal activity the organisms selected were *Aspergillus flavus*, *Aspergillus niger*, *Trichophyton mentagraphyte*, and *Candida albicans*. All the extracts were active against one or the other micro organism. Among the tested extracts, for antibacterial activity, chloroform and methanol extracts were found to be active against three of the tested organisms. Gentamycin was used as a standard. In antifungal activity, all the extracts were very much resistant against *Trichophyton mentagraphyte* but active against all other selected fungi. These results support that this common plant can be used as an antimicrobial agents.

Keywords: Antifungal, antibacterial, *Sphaeranthus amaranthoides*

INTRODUCTION

The control of microorganism is critical for the prevention and treatment of diseases. An estimate indicates that at least 5% of all persons taking an antimicrobial drug experiences some type of serious adverse reaction to it. Plants are the rich source of antimicrobial agents. There is a tremendous increase in the prevalence of pathogenic microorganisms which are resistant to the existing antibiotics. So it is necessary to find out some new anti microbial agents from natural sources which are free from side effects.

Sphaeranthus amaranthoides is a short shrub widely distributed in tropical Asia, Africa and Australia^[1-2]. It is used in treatment of chronic skin diseases and also as blood purifier. Various activities reported for the plant were protective role on dermal wounds, weak analgesic, moderate anti inflammatory activity, anti microbial, anti diarrhoeal and antioxidant activity^[3-8]. The present study is done to determine the in vitro antibacterial and antifungal property of *Sphaeranthus amaranthoides* extracts.

MATERIALS AND METHOD^[9-12]

The fresh whole plant of *Sphaeranthus amaranthoides* was collected from Thuthukudi district of Tamil Nadu. The plant was authenticated by Mr.V. Chelladurai, Retired research officer, Botany, CCRAS, Government of India.

Chemicals used

Analytical grade chemicals obtained from Merck were used for the extraction.

Selection of microorganism

The microorganisms selected for antibacterial assay are *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Shigella boydii*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila*. For antifungal assay the organisms selected are *Aspergillus flavus*, *Aspergillus niger*, *Trichophyton mentagraphyte*, and *Candida albicans*

Preparation of extract

The shade dried plant material were defatted using n-hexane and then extracted successively with solvents of increasing polarity from petroleum ether, chloroform, ethyl acetate to methanol. All the extracts were concentrated to a constant weight and stored in desiccators until use.

ANTIBACTERIAL PROPERTY**Preparation of inoculum**

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated for 24 h at 37°C. The Assay was performed by agar disc diffusion method.

Agar disc diffusion method

Antibacterial activity of plant extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium is poured in to the Petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The discs were placed in MHA plate and 20 µl of sample of varying concentration [25µg, 50 µg and 75 µg] of the plant extract was added to the disc. Standard disc contained Gentamycin (10 µg/disc). All the plates were incubated for 24 h; at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

ANTIFUNGAL PROPERTY**Preparation of Potato Dextrose Broth (PDB)**

About 3.9 g of potato dextrose agar was weighed, dissolved in 100 ml distilled water and 1 g of agar was added to this. Then, the medium is kept for sterilization.

After sterilization the media was poured in to sterile Petri plates, these Petri plates were allowed to solidify for twenty minutes. After solidification, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The discs were placed in PDA plate and 20 µl of sample of varying concentration [25µg, 50 µg and 75 µg] was added to the disc. Standard disc contained Amphotericin B (10 µg/disc). The plates were kept at room temperature. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

Table 1 shows the result of antibacterial activity. Disc diffusion method was used for the determination of antibacterial potency of the plant. All the four extracts were tested against the microorganisms namely *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Shigella boydii*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila*. All the extracts were active against one or the other micro organism. Among the tested extracts, chloroform

and methanol extracts were found to be active against three of the tested organisms. Methanolic extract was as effective as the standard gentamycin in inhibiting the growth of *Bacillus subtilis* (zone of inhibition-13 mm). Chloroform and methanol extracts were effective against *Aeromonas hydrophila*. *Vibrio parahaemolyticus* was susceptible to chloroform and ethyl acetate extracts. *Shigella boydii* was little resistant to all the extracts. Petroleum ether extract was found to be effective against both *Staphylococcus aureus* and *Salmonella typhimurium*. All the extracts showed lesser activity than the standard against all the microorganisms except *Bacillus subtilis*. But the extracts can show more activity if the concentration is increased (more than 100 µg/disc).

Table 2 shows the result of antifungal activity. All the extracts were very much resistant against *Trichophyton mentagraphyte*. Methanolic extract was as effective as the standard Amphotericin B (10 µl/disc of 100 mg/ml solution) in inhibiting *Aspergillus flavus*. Except *Trichophyton mentagraphyte*, the methanolic extract was effective against all the tested organisms namely *Aspergillus flavus*,

Aspergillus niger, and *Candida albicans* showing the zone of inhibition as 12 mm, 11 mm and 13 mm respectively.

There is a difference in susceptibility between different extracts against various organisms. This may be due to difference in cell wall composition and / or inheritance genes on plasmids that are transferred among bacterial strains and also due to difference in concentration of antimicrobial agent present in each extract. All the plants are protected themselves against pathogenic microbial infection because of the presence antimicrobial compound present in it.

Four different extracts of the plant *Sphaeranthus amaranthoides* were tested for antibacterial activity against the selected species. Among the six organisms tested all the extracts were found to be less susceptible to *Staphylococcus aureus* and *Shigella boydii*. In determination of antifungal activity, *Trichophyton mentagraphyte* was found to be resistant to all the extracts. All other fungal species were significantly inhibited by all the four extracts.

Table 1: Antibacterial activity of various extracts of *Sphaeranthus amaranthoides* showing zone of inhibition in mm

micro organism	Standard in µg/disc	petroleum ether extract in µg/disc			chloroform extract in µg/disc			ethyl acetate extract in µg/disc			methanol extract in µg/disc		
		25	50	75	25	50	75	25	50	75	25	50	75
<i>Staphylococcus aureus</i>	24	11	13	15	13	13	14	10	11	12	11	12	12
<i>Bacillus subtilis</i>	13	10	12	11	9	10	10	10	11	11	10	12	13
<i>Salmonella typhimurium</i>	15	12	12	13	10	11	11	9	10	10	10	11	12
<i>Shigella boydii</i>	25	9	10	10	10	10	11	9	9	10	10	12	12
<i>Vibrio parahaemolyticus</i>	15	9	10	11	10	11	12	10	11	12	9	10	10
<i>Aeromonas hydrophila</i>	22	9	10	10	9	11	13	9	10	11	11	12	13

Table 2: Antifungal activity of various extracts of *Sphaeranthus amaranthoides* showing zone of inhibition in mm

micro organism	Standard in µg/disc	petroleum ether extract in µg/disc			chloroform extract in µg/disc			ethyl acetate extract in µg/disc			methanol extract in µg/disc		
		25	50	75	25	50	75	25	50	75	25	50	75
<i>Aspergillus flavus</i>	12	9	10	10	7	9	10	8	9	10	8	10	12
<i>Aspergillus niger</i>	14	7	8	9	8	9	9	7	8	10	8	9	11
<i>Trichophyton mentagraphyte</i>	16	-	-	-	-	-	-	-	-	-	-	-	-
<i>Candida albicans</i>	15	10	11	12	8	8	9	9	10	11	11	12	13

CONCLUSION

These findings provide scientific evidence to support the traditional medicinal uses of these extracts and indicate a promising potential of the selected plant for medicinal purposes. Thus it can be used in the treatment of infectious diseases caused by pathogenic bacteria. Further *in vivo* studies are necessary to substantiate our findings. More importantly there is need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of plants is preserved and also to provide scientific evidence for their efficacy.

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