ANTIMICROBIAL ACTIVITY STUDY OF ETHANOLIC EXTRACT OF *ALTERNANTHERA SESSILIS* LINN. AERIAL PARTS

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The ethanolic extract of Alternanthera *sessilis* Linn. was evaluated for antimicrobial activity study against medically important gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus polymexia* & *Streotococcus faecalis*, gram negative bacteria such as *Pseudomonas aerugenosa*, *Salmonella typhii*, *Shigella dysenteriae* & *Escherichia coli* and fungi like *Penicillum notatum*, *Aspergillus niger* & *Candida albicans*. The in-vitro antimicrobial activity, minimum inhibitory concentration (MIC) of ethanolic extract was performed by broth dilution method and the zone of inhibition was studied by agar disc diffusion method at concentrations of 2, 5 and 10mg/ml in DMSO. Ciprofloxacin (5µg/ml) and Cotrimazole (25µg/ml) were used as reference control for the antibacterial and antifungal studies respectively. The results of MIC study revealed the antimicrobial activity of the extract against the tested strains of microorganisms between concentration range of 50 and 400 µg/ml. The results of zone of inhibition study revealed concentration dependant nature of the extract with better effectiveness against gram-positive bacteria than gram-negative bacteria. The present study indicates the potential usefulness of *Alternanthera sessilis* Linn. aerial parts in the treatment of various pathogenic diseases as mentioned in the ayurvedic literature.

KEYWORDS: Medicinal Plant, Ethanolic extract, Antimicrobial Activity, Bacteria, Fungus

INTRODUCTION

From the early begaing of culture plants had been a valuable source of natural medicine. It considered to be a good sources of traditional medicine which maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now a day, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [1]. The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities [2]. Herbs have been used to promote good health since ancient times. Herbal remedies use the whole plants: powdered so they can be swallowed, drunk as a tincture, decoction or infusion, or mixed with an oil-based carrier to form an ointment. In general herbal medicines have a gentle action and may take a number of weeks to achieve their effect. With herbal medicines the overall effect is usually due to combination of natural constituents which modify each other's action [3]. *Alternanthera*

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sessilis Linn. (Amaranthaceae) is an annual or perennial prostate herb with several spreading branches, bearing short petioled simple leaves and small white flowers, found throughout the hotter part of India, ascending to an altitude of 1200m [4]. The plant spreads by seeds, which are wind and water-dispersed and by rooting at stem nodes. Young shoots and leaves are eaten as a vegetable in Southeast Asia [5]. It is a weed of rice throughout tropical regions and of other cereal crops, sugarcane and bananas. Although it is a weed, it has many utilities. The leaves were used in eye diseases, cuts, wounds and antidote to snake bite; skin diseases [6]. It is also reported about the wound healing property of *Alternanthera sessilis* [7]. The degenerative and necrotic changes in the liver and kidney in Swiss mice, caused by oral administration of water extract of *A. sessilis* in high doses through histopathological test were revealed [8]. In recently, a lot of studies related to antimicrobial activities of plant extracts [9-13] and callus extracts [14-19] have been carried out.

MATERIAL AND METHODS

Plant material

The plant was identified by the Botanist of VR College, Nellore, Andhra Pradesh. After authentication the fresh aerial parts were collected from rural belt of Jangalakandriga village, Nellore, Andhra Pradesh. The plants were washed properly, shade dried and then milled to coarse powder by a mechanical grinder. The crude powder drug was kept in air tight container for further use.

Preparation of extract

The powdered plant material was defatted with petroleum ether (60-80°C) and then extracted with 95% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black coloured sticky residue (yield- 14.8% w/w on dried material basis). The dried extract was then mixed with dimethyl sulfoxide (DMSO) for antimicrobial study. Preliminary phytochemical screening [20] of the extract gave positive tests for presence of alkaloids, flavonoids, triterpenoids, glycosides, tannins, amino acids and saponins.

Drugs used

Ciprofloxacin and Cotrimazole were used as reference standards for the antibacterial and antifungal studies respectively.

Microorganism used

For the present study, the microorganisms used include *Staphylococcus aureus ATCC 25923, Bacillus subtilis UC 564, Bacillus polymexia 474, Streptococcus faecalis ATCC 29212, Pseudomonas aerugenosa 25619, Salmonella typhi 57, Shigella dysenteriae ATCC* C_3*, Escherichia coli NCTC 8196, Penicillum notatum ATCC 11625, Aspergillus niger AB 41* and *Candida albicans ATCC 18804* respectively. Suitable strains of these microorganisms were procured from the microbiology laboratory of the Institute.

ANTIMICROBIAL ACTIVITY:

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the extract was performed by broth dilution method [21] at concentrations of the extract ranging from 50 µg/ml to 400 µg/ml in DMSO against all the test microorganisms.

Determination of zone of inhibition

The zone of inhibition of the extract was performed by agar disc diffusion method [22] at concentrations of 2, 5 and 10 mg/ml of the extract in DMSO. Ciprofloxacin (5 µg/ml) and Cotrimazole (25 µg/ml) were used as reference controls for the antibacterial and antifungal studies respectively. Solvent control (only DMSO) was also maintained throughout the experiment.

RESULTS:

Table 1 depicts the antimicrobial activity of the ethanolic extract of *Alternanthera sessilis* aerial parts. The results of MIC study revealed the antimicrobial activity of the extract against the tested strains of microorganisms between concentration ranges of 50 and 400µg/ml. The results of zone of inhibition study revealed that the extract possess antimicrobial activity in a concentration dependent manner against the test organisms and was comparable with the standard drugs. The gram-positive bacteria were observed to be

Microorganisms	MIC (µg/ml)	Zone of inhibition (mm)[a]			
		Extracts (mg/ml)			Standards[b]
		2	5	10	
Gram-positive bacteria:					
Staphylococcus aureus ATCC 25923	100	8.7	17.3	19.7	27.3
Bacillus subtilis UC 564	75	9.2	17.0	20.3	25.0
Bacillus polymexia 474	75	9.5	17.8	23.3	22.3
Streptococcus faecalis ATCC 29212	150	8.3	12.7	16.7	26.7
Gram-negative bacteria:					
Pseudomonas aerugenosa 25619	150	8.5	15.0	17.7	24.3
Salmonella typhi 57	100	9.0	16.3	19.3	23.3
Shigella dysenteriae ATCC C_3	100	8.2	15.0	18.0	25.3
Escherichia coli NCTC 8196	200	7.5	12.7	15.7	21.0
Fungi:					
Penicillum notatum ATCC 11625	300	9.0	12.0	15.3	20.3
Aspergillus niger AB 41	250	8.3	13.7	17.3	23.7
Candida albicans ATCC 18804	200	9.7	17.3	20.0	28.3

Table: 1.MIC (µg/ml) and zone of inhibition (mm) of ethanolic extract of *Alternanthera sessilis* Linn. aerial parts

[a]Values are mean of three readings, [b]Standards: Antibacterial studies- Ciprofloxacin- 5µg/ml; Antifungal studies- Cotrimazole- 25µg/ml

more susceptible than gram-negative bacteria. These observations are more likely to be the fact that an outer membrane in gram negative bacteria, which acts as a barrier to many environmental substances including antibiotics [23].

DISCUSSION:

Infectious diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. The present study indicates the potential usefulness of *Alternanthera sessilis* in the treatment of various pathogenic diseases as mentioned in the Ayurvedic literature. Among the tested strains of bacteria, the extract was most effective against *Bacillus polymexia* and least against *Escherichia coli* which is naturally resistant to antibacterial agents [24]. Further study regarding the isolation and characterization of the active constituents responsible for such activity is currently under progress.

CONCLUSION:

From the study it can be concluded that the ethanolic extract of *Alternanthera sessilis* Linn. shows a

significant antimicrobial activity which may be due the presence of secondary active metabolites in the plant extracts.

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