

Antimicrobial Activity of Selected Plants Grown on Simhachalam Hills of Visakhapatnam District, Andhra Pradesh, India

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Medicinal plants have been used virtually in all cellular systems as a source of medicine. The fresh leaves of wild plants were collected from Simhachalam hills and examined for antimicrobial activity. Zone method was employed for antimicrobial assay. Methanolic leaf extracts of all the selected plants exhibited antimicrobial activity. The extracts are found to be more effective against gram-positive bacteria and gram-negative bacteria (Zone size: 9 to 27 mm including well size). Annonaceae member (*Polyalthia longifolia*) has shown good result against selected bacteria (21 to 26 mm). Apocyanaceae member (*Vinca alba*) has shown least activity against bacteria.

Key words: Wild plants, Antimicrobial activity, Simhachalam hills.

To define and describe the future tasks of phytomedicinal research in the new millennium, an analysis not only of the current state of development of phytomedicinal research but also of chemosynthetic pharmaceutical research. One advantage of phytotherapy is the

availability of a wide group of medicinal drugs and preparation's that have been used over the centuries almost exclusively on the basis of empirical evidence. A reservoir of around 3,00,000 plant species exists, of which only about 30 percent have been investigated scientifically, inclusively the herbs and preparations of Indian, Chinese, South American and African traditional medicines¹.

Various antimicrobial studies are conducted using methanolic leaf extracts from various plants during past decades. The reports of plant extracts *Caesalpinia pulcherrima*, *Euphorbia hirta* and *Casuarina equisetifolia* were shown most active against Gram-positive bacteria *B. cereus*², *Mitracarpus scaber*. leaves formulated at a minimum inhibitory concentration of 75mg/ml on *E.coli*³, *R. tetraphylla* and *P. minima*

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inhibited bacterial and fungal growth using methanol extract showed MIC of 0.25 to 100 mg/ml against bacterial pathogens and 0.5 to 100 mg/ml against fungal pathogens⁴.

Medicinal herbs are moving from fringe to mainstream use as a greater number of people endeavor to opt for herbal formulations over the allopathic compounds, since these are devoid of side effects and cost effective⁵. The earlier examples of western medicine had the influence of plant medicines used in ancient times⁶.

The importance of plants extracts can be used as a potential source of antibiotics controlling various fungal and bacterial pathogens⁷. The antimicrobial qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s^{8,9}. Chemotherapeutic agents used orally or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious disease. The tested plant products appear to be effective against a wide spectrum of microorganisms, both pathogenic and nonpathogenic. Administered orally, these compounds may be able to control a wide range of microbes but there is also the possibility that they may cause an imbalance in the gut microflora, allowing opportunistic pathogenic coliforms to become established in the gastrointestinal tract with resultant deleterious effects^{10,11}.

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins,

terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties^{12,13}.

MATERIAL AND METHODS

Collection of plant materials

The plants are widely distributed in Simhachalam hills of Visakhapatnam District. Healthy leaves are collected and were used for experiment.

Preparation of plant extracts

Fresh leaves were separately washed thoroughly under running water, shade dried and used for extraction. The plant materials were homogenized to a fine powder and stored in airtight bottles.

The methanolic extracts of plants were extracted using soxhlet extractor. About 25 g of leaf powders separately were carefully transferred into round bottom flask of soxhlet extractor. The plant materials were soaked in 2 litres of methanol for 24 hours at room temperature. The final extracts were filtered through whatman's filter paper no.1. The methanol present in the methanolic extract was evaporated under reduced pressure (Buchi vaporator) to yield the residue. The residue thus obtained was suspended in DMSO (Dimethylsulfoxide) to obtain different concentrations of crude extract. These extracts were evaluated for their antimicrobial activity using zone method.

Microorganisms and their maintenance

Four bacterial and two fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and preserved in deep freezer at Department of Biotechnology, VS Krishna PG College, Visakhapatnam, India. The cultures employed for experimentation are *Pseudomonas aeruginosa* (MTCC 2488), *Escherichia coli* (MTCC 118), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 96), *Micrococcus leuteus* (MTCC 106), *Streptococcus faecalis* (MTCC 459) and *Lactobacillus plantarum* (MTCC 1407).

The above bacterial cultures were maintained on Muller Hinton Agar (MHA) at 4°C temperature until used for the study. Before use, the bacterial cultures were revived in Muller Hinton Broth (MHB).

Procedure for Antimicrobial assay (Zone method)

MHA was weighed and mixed in distilled water based on the composition. The media was autoclaved for 20 minutes at 121°C (15 lbs pressure) and cooled to 45°C. The bacterial cultures with optical density of 0.6 were taken and 50 ml of inoculum was added per 500ml of MHA. To each petriplate, 20 ml of the media was poured and was kept for solidification. By using gel puncture (8 mm diameter) wells have been made in the plate for the addition of plant extracts. The prepared plant extracts with concentration of 0.5 mg/ml were tested separately for their antimicrobial activity

After addition of the plant extracts in agar wells, the plates were kept aside for 2 hours for diffusion. Bacterial cultures are incubated for 18 hours at 37°C. The result was obtained by measuring the zone diameter, an indication of growth of the microorganisms. The experiment was repeated three times and the mean values are presented.

RESULTS

The ethnobotanical screening tests of wild leaf extracts of Ashoka, Polyalthia, Aristolochia, Rauvolfia, Ganneru, Karakkaia, Coccinia, Catharanthus, Oxalis, Alamanda,

Table 1. Antimicrobial activity of Methanolic leaf extracts of wild plants (concentration of 0.5mg/ml) (Zone of activity in mm including 8mm Well diameter)

Scientific Name/Family	Common Name	PA g-	EC g-	BS g+	SA g+	ML g+	SF g+	LP g+
<i>Saraca asoca</i> / Fabaceae	Ashoka	13	11	12	19	12	13	08
<i>Polyalthia longifolia</i> / Annonaceae	Polyalthia	26	24	21	27	21	21	21
<i>Aristolochia macrophylla</i> / Aristolochiaceae	Aristolochia	14	15	19	12	13	13	13
<i>Rauvolfia serpentina</i> / Asclepiadaceae	Rauvolfia	11	13	18	16	9	9	9
<i>Vinca alba</i> / Apocyanaceae	Ganneru	-	12	9	9	9	-	-
<i>Terminalia chebula</i> / Combretaceae	Karakkaia	18	11	19	20	18	18	19
<i>Coccinia grandis</i> / Cucurbitaceae	Coccinia	12	13	11	11	9	9	12
<i>Catharanthus roseus</i> / Apocynaceae	Catharanthus	12	12	14	13	12	11	11
<i>Oxalis stricta</i> / Oxalidaceae	Oxalis	13	14	17	18	11	13	12
<i>Allamanda cathartica</i> / Apocynaceae	Alamanda	14	16	14	14	13	13	12
<i>Gomphrena globosa</i> / Amaranthaceae	Gomphrena	17	17	16	17	16	14	14
<i>Ocimum basilicum</i> / Lamiaceae	Basil	18	18	15	16	15	15	15
Standards used								
Penicillin			18	14				
Streptomycin			17	26				

Note: *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Micrococcus leuteus* (ML), *Streptococcus faecalis* (SF) and *Lactobacillus plantarum* (LP).
g- = gram negative; g+ = gram positive

Gomphrena and Basil in methanol as solvent against both human and plant pathogenic bacteria using zone technique are depicted in Tables 1. Two antibiotics (Penicillin and Streptomycin) were used as standards and the zone of inhibition was shown to be 14mm (*B. subtilis*), 18mm (*E. coli*) with penicillin and 26mm (*B. subtilis*), 17 mm (*E. coli*) with streptomycin and were listed in Table 1.

Saraca asoca

Methanolic extracts of the leaf parts of *Saraca asoca* have exhibited antimicrobial activity. Zone of inhibition (in mm) of *Saraca asoca* against selected bacteria was reported as *Pseudomonas aeruginosa* (13), *Escherichia coli* (11), *Bacillus subtilis* (12), *Staphylococcus aureus* (19), *Micrococcus leuteus* (12), *Streptococcus faecalis* (13) and *Lactobacillus plantarum* (08). *Saraca asoca* leaf extracts has shown good activity against Gram-positive bacteria.

Polyalthia longifolia

Zone of inhibition for *Polyalthia longifolia* against selected bacteria was reported as - *Pseudomonas aeruginosa* (26), *Escherichia coli* (24), *Bacillus subtilis* (21), *Staphylococcus aureus* (27), *Micrococcus leuteus* (21), *Streptococcus faecalis* (21) and *Lactobacillus plantarum* (21).

Aristolochia macrophylla

Zone of inhibition for *Aristolochia macrophylla* against selected bacteria was reported as - *Pseudomonas aeruginosa* (14), *Escherichia coli* (15), *Bacillus subtilis* (19), *Staphylococcus aureus* (12), *Micrococcus leuteus* (13), *Streptococcus faecalis* (13) and *Lactobacillus plantarum* (13).

Rauvolfia serpentina

Zone of inhibition for *Rauvolfia serpentina* against selected bacteria was reported as - *Pseudomonas aeruginosa* (11), *Escherichia coli* (13), *Bacillus subtilis* (18), *Staphylococcus aureus* (16), *Micrococcus leuteus* (9), *Streptococcus faecalis* (9) and *Lactobacillus plantarum* (9).

Vinca alba

Vinca alba did not shown good antimicrobial property.

Terminalia chebula

Terminalia chebula has shown good antimicrobial property against all the selected

bacteria (11 to 20 mm)

Coccinia grandis

Coccinia grandis has shown moderate activity (9mm to 13mm zone size) against selected microbes.

Catharanthus roseus

Catharanthus roseus has shown moderate activity (11mm to 14mm zone size) against selected microbes.

Allamanda cathartica

Zone of inhibition (in mm) for *Allamanda cathartica* against selected bacteria was reported as - *Pseudomonas aeruginosa* (14), *Escherichia coli* (16), *Bacillus subtilis* (14), *Staphylococcus aureus* (14), *Micrococcus leuteus* (13), *Streptococcus faecalis* (13) and *Lactobacillus plantarum* (12).

Gomphrena globosa

Zone of inhibition (in mm) for *Gomphrena globosa* against selected bacteria was reported as - *Pseudomonas aeruginosa* (17), *Escherichia coli* (17), *Bacillus subtilis* (16), *Staphylococcus aureus* (17), *Micrococcus leuteus* (16), *Streptococcus faecalis* (14) and *Lactobacillus plantarum* (14).

Ocimum basilicum

Zone of inhibition (in mm) for *Ocimum basilicum* against selected bacteria was reported as - *Pseudomonas aeruginosa* (18), *Escherichia coli* (18), *Bacillus subtilis* (15), *Staphylococcus aureus* (16), *Micrococcus leuteus* (15), *Streptococcus faecalis* (15) and *Lactobacillus plantarum* (15).

DISCUSSION

The potential for developing antimicrobials from plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. India is a varietal emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for

introducing and domesticating new exotic plant varieties^{14,15,16}.

Annonaceae member (*Polyalthia longifolia*) has shown good results against selected bacteria (21 to 26 mm). Apocyanaceae member (*Vinca alba*) has shown least activity against selected bacteria.

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