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Studies on In-vitro antimicrobial activity of Some Medicinal Plants from Visakhapatnam

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ABSTRACT

In this present study seven terrestrial plants was screened for antimicrobial activity against some clinical and phytopathogens. The plants were collected from surroundings of Visakhapatnam, dried and extracted successively with hexane, chloroform and methanol using the soxhlet extraction apparatus. The antimicrobial activities of the plant extract on the various test microorganisms, including multiple antibiotic resistant bacteria, were investigated. The plants have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

Keywords: Antibiotic resistant bacteria, antimicrobial activity.

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INTRODUCTION

World is endowed with a rich wealth of medicinal plants and man cannot survive on this earth for long healthy life without the plant kingdom because, the plant products and their active constituents played an important role in maintaining perfect health. Herbal medicine is the world's most ancient form of medicine as it is evident from the fact that every ancient civilization used plants for healing and in many cultures, herbal knowledge was said to have been handed down from the God. Plants have always been the principle for of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in count with the body's own defense [1] There is a wide spread belief that green medicines are healthier and more harmless or safer than synthetic one.

Literature indicates that various investigators have studied antimicrobial studies in recent past [2-7] in *Vinkarosea*[8] *Cassia alanta* and *Lawsonia alba* [9], and Tea leaf [10] Seedling juice of *Ipomoea cornea* showed fungi static properties [11], Larvicide [12] and antimicrobial activity have been reported [13,14] studied the crude fractions of *calotropisprocera* flower bud and root against a chloroquine sensitive strain MRC 20 and chloroquine resistant strain MRC 76 of *plasmodium fulcifarum* and the effectiveness of its fractions compare better with CQ sensitive strain than theCQ resistant strain in *In-vitro*.

MATERIALS AND METHODS

The seven plant species used for the present study were collected from the Botanical Garden of the Department of Botany and surrounding areas of the Andhra University. The selected plant parts were thoroughly washed and dried under shade of $28 \pm 2^{\circ}\text{C}$ for about 10 days. The dried plant samples were ground well into a mixer grinder and sieved to give particle size of 50-150 mm. The powders were stored in air sealed polythene bags at room temperature before extraction.

Table-1: Details of the Microorganisms used in Bioassay

| Name of the microorganism | Abbreviation | MTCC Catalogue No. | Culture type |
|------------------------------------------------------------|--------------|--------------------|--------------|
| <i>Staphylococcus aureus</i> | SC | 7405 | ATCC 25923 |
| <i>Bacillus subtilis</i> | BC | 7352 | ATCC 21332 |
| <i>Streptococcus</i> | S | 6163 | ATCC 11988 |
| <i>Pseudomonas aureginosa</i> | PA | 7903 | ATCC 27853 |
| <i>Escherichia coli</i> | EC | 4296 | ATCC 25922 |
| <i>Saccharomyces cerevisiae</i> <i>Aspergillusniger</i> | SC | 463 | ATCC 834 |
| | AN | 2425 | ATCC 9142 |



Extracts Preparation

Dried powder (20g) of required part of plant was packed in a Whatmann filter paper no.1 and was extracted in a Soxhlet apparatus using 100ml of solvents used for extraction were Methanol (78.5C), Chloroform (61C) and Water (80C) as solvent and the extracts were stored in a refrigerator at 4C.

Seven plant species were collected from the Andhra University Campus and in around the Visakhapatnam Description of the plant material and medicinal uses.

Boerhaaviadiffusa(Linn), Family:Nyctaginaceae

A variable, procumbent herbs 50-70 cm long, leaves-ovate-sub elliptic, flowers-pale pink in paniculate umbels and fruit-glandular and oblong.

Flower and Fruit: Punarnava or Ambatimadu

Distribution: A common weed in cultivated fields and forest outskirts. It is growing in divers habits from coastel regions to hilly areas up to + 750 altitudes.

Sapindusemerginatus (Vahl), Family: Sapindaceae

A moderate sized tree, leaves-abruptly pinnate, leaflets entire oblong, emarginated apex, flowers- brownish- yellow, terminal panicles and ovoid -3 lobed and fruits- drupes.

Flowering: November- January

Local name: Kunkudu and Rittachettu.

Fruiting: February – April

Distribution: Frequent near villages and rare in the forest areas.

Pedaliium murex(Linn), Family: Pedaliaceae.

Glabrous herbs, stem- angular, leaves- long, broadly ovate- oblong, opposite, flowers – golden yellow axillary, solitary and fruit – long, pericarp thorny.

Flowering and fruiting: July –October.

Distribution: Common in waste lands throughout the state.

Cleome viscosa (Linn), Family: Capparidaceae.

An erect, viscous, glandular herb up to 50 – 90 cm high, leaves – very sticky, leaves 3 -5 foliate, leaflets-elliptic – oblong or obovate.

Flowers- racemes, corolla –Yellow,

Capsule – thinly glandular up to 6 cm long.

Flowering and fruits: July –December.

Local name: Kukkavaminta.

Distribution: A very common weed of waste lands and open fields and road sides.

Ruelliatuberosa (Linn), Family: Acanthaceae.

Erect herbs about 20cm height, leaves – ovate elliptic, acute at apex and undulate – crenate, flowers –blue in axillary cymes, capsules –long, linear.

Flowering and fruits: September –November. Local name: Anantharam

Distribution: An annual weed of open places during rainy seasons both a long forest paths and rice fields.

Cassia occidentalis (Linn), Family: Caesalpiniaceae.

Erect herb-under shrub, Leaflets- 4-5 pairs, ovate-lanceolate, flowers – Yellow long in axillary racemes and pods flat.

Local name: Kasinda

Distribution: Abundant along weed road sides, in forest outskirts, in open Lands and along railway lines in the district.

Aervalanata(Juss.), Family: Amaranthaceae.

Erect – sub erect herbs, leaves –obovate - oblong, flowers- whitish in axillary cylindric, utricles indehiscent and seeds – black in colour.

Flowering and fruits: August – November

Local name: Pindikura

Distribution: A frequent weed in cultivated fields, outskirts of forests and near villages in hilly areas and also delta and coastal areas of the district.

RESULTS

Screening of Antibacterial and Antifungal activity (Inhibition zones)

Plant extracts (Methanol, Chloroform and Water) of seven commonly occurring weeds namely *Boerhaaviadiffusa*, *Sapindusemerginatus*, *Pedaliium murex*, *Cleome viscose*, *Ruellia tuberosa*, *Cassia occidentalis* and *Aervalanata* for their antimicrobial effects were tested against five selected bacteria viz., *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus* (Gram positive) *Pseudomonas aureginosa*, *Escherichia coli* (Gram negative) and two fungal species *Saccharomyces cerevisiae* and *Aspergillus niger* Through agar diffusion method.

Antimicrobial activity of plants

Antibacterial activity of methanol extracts of different plants

Antibacterial activities of methanol extracts of whole plant of selected plants were observed using the disc diffusion method by measuring the diameter of the growth inhibition zones and Activity Index. The mean values of inhibition zones and Activity index are depicted in Table-2. The methanol extract of whole plant of *Boerhaaviadiffusa* showed high degree of inhibition against *Bacillus subtilis* but none of the other bacterial strains were affected. The extracts of *Sapindusemerginatus* and *Pedaliium murex* were not showed any activity against any of the bacterial strains. The extract of *Cleome viscosa* showed inhibitory activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and none of the other bacterial strains were affected. The methanol extract of *Ruellia tuberosa* showed inhibitory activity against *Streptococcus*, *Pseudomonas aureginosa*, and *Escherichia coli* except *Staphylococcus aureus* and *Bacillus subtilis*. The extract of *Cassia occidentalis* showed inhibitory activity against only *Staphylococcus aureus* and *Bacillus subtilis* and none of the other bacterial strains were affected. The extract of *Aervalanata* did not showed any activity against the bacterial strains.

Table 2 Antibacterial Activity of Methanolic Extract of Different Plant Extracts

| S No | Name of the Plant/antibiotic | Tested Bacteria | | | | | | | | | |
|------|------------------------------|------------------------------|------|--------------------------|------|----------------------|------|-------------------------------|------|-------------------------|------|
| | | <i>Staphylococcus aureus</i> | | <i>Bacillus subtilis</i> | | <i>Streptococcus</i> | | <i>Pseudomonas aureginosa</i> | | <i>Escherichia coli</i> | |
| | | IZe | AI | IZe | AI | IZe | AI | IZe | AI | IZe | AI |
| 1 | <i>Boerhaaviadiffusa</i> | - | - | 10±0.63 | 0.62 | - | 0.56 | - | - | - | - |
| 2 | <i>Sapindusemargi natus</i> | - | - | - | - | - | - | - | - | - | - |
| 3 | <i>Pedaliium murex</i> | - | - | - | - | - | - | - | - | - | - |
| 4 | <i>Cleome viscosa</i> | 10±0.36 | 0.49 | 11±0.54 | 0.40 | - | - | - | - | 10±0.4 | 0.45 |
| 5 | <i>Ruelliatuberosa</i> | - | - | - | - | 11±0.63 | 0.68 | 10±0.49 | 0.50 | 10±0.63 | 0.45 |
| 6 | <i>Cassia occidentalis</i> | 15±0.63 | 0.55 | 11±0.63 | 0.68 | - | - | - | - | - | - |
| 7 | <i>Aervalanata</i> | - | - | - | - | - | - | - | - | - | - |
| 8 | <i>DMSO</i> | - | - | - | - | - | - | - | - | - | - |
| 9 | <i>Tetracycline</i> | 20±0.45 | - | 16±0.63 | - | 16±0.49 | - | 20±0.67 | - | 22±0.49 | 0.40 |

Antibacterial activity of Chloroform extracts of plants

Antibacterial activities of chloroform extracts of different plants were observed using the disc diffusion method by measuring the diameter of the growth inhibition zone and activity index. The mean growth inhibition zone and activity index are given in Table-3. The extract of *Boerhaaviadiffusa* showed moderate inhibition against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aureginosa* but no inhibitory effects against *Streptococcus* and *Escherichia coli*. The *Sapindusemargina tus* extracts showed moderate inhibition zone against all the studied bacterial strains except *Bacillus subtilis*. The extract of *Pedaliium murex* showed inhibitory activity only *Staphylococcus aureus* and *Pseudomonas aureginosa*. The extracts of *Cleome viscosa* showed moderate inhibitory zone against all the five bacterial strains. The extract of *Ruellia tuberosa* was found to be active against *Streptococcus*, *Pseudomonas aureginosa* and *Escherichia coli*. *Cassia occidentalis* had activity against almost all the microbes except *Streptococcus*. The extract of *Aervalanata* showed moderate inhibition activity against *Bacillus subtilis* and *Pseudomonas aureginosa*.

Table 3 Antibacterial Activity of Chloroform Extract of Different Plant Extracts

| S No | Name of the Plant/antibiotic | Tested Bacteria | | | | | | | | | |
|------|------------------------------|------------------------------|------|--------------------------|------|----------------------|------|-------------------------------|------|-------------------------|------|
| | | <i>Staphylococcus aureus</i> | | <i>Bacillus subtilis</i> | | <i>Streptococcus</i> | | <i>Pseudomonas aureginosa</i> | | <i>Escherichia coli</i> | |
| | | IZe | AI | IZe | AI | IZe | AI | IZe | AI | IZe | AI |
| 1 | <i>Boerhaaviadiffusa</i> | 10±0.49 | 0.37 | 12±0.63 | 0.75 | - | - | 10±0.40 | 0.50 | - | - |
| 2 | <i>Sapindusemargina tus</i> | 12±0.49 | 0.44 | - | - | 10±0.49 | 0.62 | 12±0.40 | 0.60 | 10±0.63 | 0.45 |
| 3 | <i>Pedaliium murex</i> | 10±0.27 | 0.37 | - | - | - | - | 10±0.45 | 0.50 | - | - |
| 4 | <i>Cleome viscosa</i> | 13±0.40 | 0.48 | 12±0.63 | 0.75 | 10±0.63 | 0.62 | 13±0.28 | 0.65 | 10±0.54 | 0.45 |
| 5 | <i>Ruelliatuberosa</i> | - | - | - | - | 10±0.49 | 0.62 | 12±0.28 | 0.60 | 10±0.49 | 0.45 |
| 6 | <i>Cassia occidentalis</i> | 12±0.27 | 0.44 | 10±0.49 | 0.62 | - | - | 11±0.49 | 0.55 | 12±0.28 | 0.54 |
| 7 | <i>Aervalanata</i> | - | - | 12±0.63 | 0.75 | - | - | 10±0.54 | 0.50 | - | - |
| 8 | <i>DMSO</i> | - | - | - | - | - | - | - | - | - | - |
| 9 | <i>Tetracycline</i> | 20±0.45 | - | 16±0.63 | - | 16±0.49 | - | 20±0.67 | - | 22±0.49 | - |

Antibacterial activity of water extracts of different plants

Antibacterial activities of water extracts of different plants were studied by measuring the diameter of the growth inhibition zone and Activity Index. The mean values of inhibition zones and Activity index are depicted in Table-4. The water extract of *Boerhaaviadiffusa* showed inhibitory activity against *Streptococcus* only and none of the other bacterial strains

were affected. The extracts of *Sapindusemerginatus* and *Cleome viscosa* were not showed any inhibitory activities against all of the five bacterial strains. The extract of *Pedaliium murex* showed moderate inhibitory activity against *Pseudomonas aureginosa* only and none of the other bacterial strains were affected. The extract of *Ruellia tuberosa* showed inhibitory activity against *Staphylococcus aureus* and *Bacillus subtilis* only. Similarly the extracts of *Cassia occidentalis* showed moderate inhibitory activity against *Streptococcus* and *Pseudomonas aureginosa* and none of the other bacterial strains were affected. The water extract of *Aervalanata* showed moderate inhibitory activity against all the selected bacterial strains except *Bacillus subtilis*.

Table 4 Antibacterial Activity of Water Extracts of Different Plant Extracts

| S No | Name of the Plant/antibiotic | Tested Bacteria | | | | | | | | | |
|------|------------------------------|------------------------------|------|--------------------------|------|----------------------|------|-------------------------------|------|-------------------------|------|
| | | <i>Staphylococcus aureus</i> | | <i>Bacillus subtilis</i> | | <i>Streptococcus</i> | | <i>Pseudomonas aureginosa</i> | | <i>Escherichia coli</i> | |
| | | Ize | AI | Ize | AI | Ize | AI | Ize | AI | Ize | AI |
| 1 | <i>Boerhaaviadiffusa</i> | - | - | - | - | 12±0.49 | 0.75 | - | - | - | - |
| 2 | <i>Sapindusemarginatus</i> | - | - | - | - | - | - | - | - | - | - |
| 3 | <i>Pedaliium murex</i> | 12±0.49 | 0.44 | - | - | - | - | - | - | - | - |
| 4 | <i>Cleome viscosa</i> | - | - | - | - | - | - | 10±0.54 | 0.50 | - | - |
| 5 | <i>Ruelliatuberosa</i> | 15±0.36 | 0.55 | 11±0.63 | 0.68 | - | - | - | - | - | - |
| 6 | <i>Cassia occidentalis</i> | - | - | - | - | 10±0.49 | 0.62 | 10±0.63 | 0.50 | - | - |
| 7 | <i>Aervalanata</i> | 12±0.49 | 0.27 | - | - | 12±0.49 | 0.75 | 10±0.49 | 0.50 | 11±0.49 | 0.50 |
| 8 | <i>DMSO</i> | - | - | - | - | - | - | - | - | - | - |
| 9 | <i>Tetracycline</i> | 20±0.45 | - | 16±0.63 | - | 16±0.49 | - | 20±0.67 | - | 22±0.49 | - |

Antifungal activity of methanol extracts of different plants

Antifungal activities of methanol extracts of different plants were studied using the disc diffusion method by measuring the diameter of the growth inhibition zone and Activity index. The mean values of inhibition zones and Activity index are depicted in Table-5. The methanol extracts of *Boerhaaviadiffusa* showed moderate activity against *Saccharomyces cerevisiae* but no inhibitory affect against *Aspergillusniger*. The extracts of *Sapindusemerginatus* were found to be no inhibitory activity against two fungal selected strains. The extracts of *Cassia occidentalis* were showed moderate inhibitory activity against two fungal strains. Similarly, the extracts of *Cleome viscosa*, *Ruellia tuberosa* and *Aervalanata* showed moderate inhibitory activity against *Aspergillusniger* only but no inhibitory affect against *Saccharomyces cerevisiae*.

Table 5 Antifungal Activity of Methanol Extracts of Different Plant Extracts

| S.No. | Name of the Plant/antibiotic | Tested Fungi | | | |
|-------|------------------------------|---------------------------------|------|-------------------------|------|
| | | <i>Saccharomyces cerevisiae</i> | | <i>Aspergillusniger</i> | |
| | | IZe | AI | IZe | AI |
| 1 | <i>Boerhaaviadiffusa</i> | 11±0.49 | 0.45 | - | - |
| 2 | <i>Sapindusemarginatus</i> | - | - | - | - |
| 3 | <i>Pedanium murex</i> | 11±0.63 | 0.45 | - | - |
| 4 | <i>Cleome viscosa</i> | - | - | 10±0.36 | 0.43 |
| 5 | <i>Ruelliatuberosa</i> | - | - | 12±0.49 | 0.52 |
| 6 | <i>Cassia occidentalis</i> | 10±0.27 | 0.50 | 11±0.40 | 0.47 |
| 7 | <i>Aervalanata</i> | - | - | 10±0.27 | 0.43 |
| 8 | <i>DMSO</i> | - | - | - | - |
| 9 | <i>Nystatin</i> | 20±0.49 | - | 23±0.63 | - |

Chloroform extracts of different plants

Antifungal activities of chloroform extracts of 7 different plant materials were studied and the mean values of inhibition zones and Activity index are depicted in Table-6. The extracts of *Boerhaaviadiffusa* and *Aervalanata* were not showed any inhibitory activity against the two fungal strains. Similarly, the extracts of *Pedanium murex* were showed inhibitory activity ranging from 10-27 mm against *Aspergillusniger* only but not *Saccharomyces cerevisiae*. The extracts of *Sapindusemerginatus*, *Ruellia tuberosa* and *Cassia occidentalis* were showed moderate activity against *Saccharomyces cerevisiae* only but no inhibitory effects against *Aspergillusniger*. The extracts of *Cleome viscosa* were showed moderate inhibition zone against both *Saccharomyces cerevisiae* and *Aspergillusniger*.

Table 6 Antifungal activity of Chloroform Extracts of different Plants

| S.No. | Name of the Plant/antibiotic | Tested Fungi | | | |
|-------|------------------------------|---------------------------------|------|-------------------------|------|
| | | <i>Saccharomyces cerevisiae</i> | | <i>Aspergillusniger</i> | |
| | | IZe | AI | IZe | AI |
| 1 | <i>Boerhaaviadiffusa</i> | - | - | - | - |
| 2 | <i>Sapindusemarginatus</i> | 11±0.28 | 0.55 | - | - |
| 3 | <i>Pedaliium murex</i> | - | - | 13±0.50 | 0.56 |
| 4 | <i>Cleome viscosa</i> | 10±0.49 | 0.50 | 10±0.49 | 0.43 |
| 5 | <i>Ruelliatuberosa</i> | 12±0.59 | 0.60 | - | - |
| 6 | <i>Cassia occidentalis</i> | 10±0.45 | 0.50 | - | - |
| 7 | <i>Aervalanata</i> | - | - | - | - |
| 8 | <i>DMSO</i> | - | - | - | - |
| 9 | <i>Nystatin</i> | 20±0.49 | - | 23±0.63 | - |

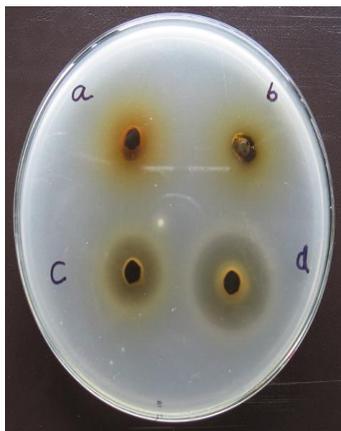
Water extracts of different plants

Antifungal activities of water extracts of different plants were studied and the mean values of inhibition zones and activity index are depicted in table -7. The water extracts of *Boerhaaviadiffusa*, *Sapindusemerginatus*, *Pedaliium murex* *Ruellia tuberosa* and *Cassia occidentalis* were found to be no inhibitory activity against the both fungal strains. Similarly, the extracts of *Aervalanata* were showed moderate inhibition zones against *Saccharomyces cerevisiae* only but inhibitory effects against *Aspergillusniger*. Likewise, the extracts of *Cleome viscosa* were showed high degree activity against *Aspergillusniger* only but inactive against *Saccharomyces cerevisiae*.

Table 7 Antifungal activity of Water Extracts of different Plants

| S. No. | Name of the Plant/antibiotic | Tested fungi | | | |
|--------|------------------------------|---------------------------------|----|-------------------------|----|
| | | <i>Saccharomyces cerevisiae</i> | | <i>Aspergillusniger</i> | |
| | | IZe | AI | IZe | AI |
| 1 | <i>Boerhaaviadiffusa</i> | - | - | - | - |
| 2 | <i>Sapindusemarginatus</i> | - | - | - | - |
| 3 | <i>Pedaliium murex</i> | - | - | - | - |
| 4 | <i>Cleome viscosa</i> | - | - | 10±0.54 | - |
| 5 | <i>Ruelliatuberosa</i> | - | - | - | - |

| | | | | | |
|---|----------------------------|---------|------|---------|---|
| 6 | <i>Cassia occidentalis</i> | - | - | - | - |
| 7 | <i>Aervalanata</i> | 10±0.49 | 0.50 | - | - |
| 8 | DMSO | - | - | - | - |
| 9 | <i>Nystatin</i> | 20±0.49 | - | 23±0.63 | - |



DISCUSSION

In present study a variety of Gram-positive, Gram-negative and fungal strains were selected for the antimicrobial effect of 7 selected common weed and medicinal plant extracts to perceive the antimicrobial spectrum as well as to authentic ethno medicinal claims. The demonstration of antimicrobial activity against bacterial and fungal strains may be indicative of the presence of board spectrum antibiotic plants. The whole plant extracts more effective than the aerial parts like leaf, flower, fruit and seed extracts. Out of the three solvents used for the extraction the methanolic extracts showed the highest activity against the test organisms followed by chloroform extracts and aqueous (water) extracts.

The methanolic plant extracts of *Boerhaaviadiffusa*, *Cleome viscosa*, *Ruellia tuberosa*, *Cassia occidentalis* showed antibacterial activities (both Gram-positive and Gram-negative) compared to their response to antibiotic tetracycline. The methanolic extracts of *Sapindusemerginatus*, *Pedaliium murex* and *Aervalanata* did not show any inhibitory activity against of the five bacterial strains. Chloroform extracts of *Cleome viscosa*, *Cassia occidentalis*, and *Aervalanata* showed moderate inhibitory activity against four selected bacterial strains except *streptococcus*. It can be concluded from the present findings plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used by resistant microorganisms. The differentiating activities against variety of microorganisms of 7 plant extracts encourage developing a novel broad spectrum antimicrobial formulation in future.

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