Phytochemical screening and antibacterial activity of 
*Hemionitis arifolia* (Burm.) Moore

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*Hemionitis arifolia* (Burm.) Moore of family Hemionitidaceae is one of the endemic and widely distributed species on Tirumala hills of Tirupati, Andhra Pradesh, India. Ethnomedicinally, the genus is important and popularly known as Ramabanum. It has been used in burns, menstrual disorders, anti-flatulence and antifertility. Eight different organic solvents were used to extract the bioactive compounds from the whole plant to screen the phytochemical and antibacterial activity against infectious disease causing bacterial pathogens such as *Enterobacter aerogens*, *Klebsiella pneumoniae*, *Salmonella paratyphi A*, *Bacillus cereus*, *Salmonella typhi*, *Salmonella paratyphi B*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus sphericus*, *Bacillus sterothermophilus* and *Micrococcus luteus* by agar well diffusion method. Phytochemical screening showed the presence of flavonoid, steroids and glycosides along with reducing sugar in all the extracts investigated. Gram-negative bacteria such as *Salmonella typhi*, *S. paratyphi A* and *Enterobacter aerogens* were more susceptible to the crude extracts than Gram-positive bacteria. Hence, at any rate *Hemionitis arifolia* is an attractive material for further research leading to possible drug development.

**Key words:** *Hemionitis arifolia*, Phytochemical screening, Antibacterial activity, Tirumala hills.

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**Introduction**

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. In industrialized nations at present, some 50% of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine species, plants and microorganisms¹. Among the estimated 250,000 plant species existing world wide, only a small percentage have been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller India’s greatest natural treasure is its medicinal and aromatic herbs, which have been used in medicine and perfumery since time immemorial. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being and the bioprospecting of new plant derived drugs. The World Health Organization has compiled a list of more than 21,000 plant species are supposedly used globally in medicine.

Now-a-days more and more Angiospermic plants are being used as medicine for many diseases. However, the lower group of plants like pteridophytes is largely neglected and has not been well documented although they are known for their beauty elegance and infinite variety. Pteridophytes which include the ferns and fern allies are a group of vascular plants of ancient or primitive land plants with worldwide distribution. The medicinal qualities of ferns, real or imaginary, are mentioned as early as 300 B.C. by the Greek, philosopher Theophrastus and his Indian contemporaries Sushrut and Charak. On the basis of preliminary checklist of pteridophytes²⁻⁴ it has been reported that within present day political boundaries of India about 191 genera comprising over 1250 pteridophytes exist.

Though recent ethnobotanical, pharmacological and biological searches have revealed medicinal, pharmaceutical and phytochemical attributes of pteridophytes, which have valuable potential applications for health and industry, still many species of pteridophytes are yet to be explored for their potential applications for future use and to isolate new active principles from them. Hence, the present study is carried out on *Hemionitis arifolia* (Burm.) Moore to

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screen phytochemicals and antibacterial activity. This species has been reported to be endemic fern and occurs on Tirumala Hills of Tirupati, Andhra Pradesh, India.

Materials and Methods

Description and ethnobotanical uses

The plant used in the present study belongs to Pteridophyta, and family Hemionitidaceae. The plant is small herbaceous fern with dark green leaves, blackish stipe and cordiform leathery leaves, an endemic and very common on the stone-walls, rock crevices in densely shaded localities with high atmospheric humidity. The fronds are two types. The sterile fronds are small. The fertile fronds are long stalked, large pinnately lobed (Plate 1). Leaves are in heart shaped hence called as heart fern/valentine fern. Sporangia occur all over the surface of the veins, forming reticulate patterns on the lower surface. The fronds are used in the treatment of aches and as vermifuge. Rhizome has antibacterial activity and fronds are used in burns, menstrual disorders, anti-flatulence and antifertility. The decoction of the whole plant is administered to cure fever.

Collection and processing

The plants were collected from Tirumala hills of Tirupati, Andhra Pradesh, India. The plant was identified as *H. arifolia* (Burm.) Moore using the Herbarium specimens at Botany Department, Sri Venkateswara University, Tirupati and maintained in departmental herbarium. All specimens were washed with sterile distilled water, cut in to small pieces and dried in shade and made into fine powder using blender. This powder was used for extraction of bioactive compounds.

Extraction

Ninety grams of powder was extracted in Soxhlet apparatus with different solvents (1000 ml each) based on their polarity, viz. petroleum ether, hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol and methanol. These extracts were concentrated using rotary evaporator at 40°C and moisture was removed by placing in desiccators and were stored in screw cap vials at 4°C until use.

Phytochemical screening

Screening of phytochemicals such as alkaloids, flavonoids, steroids, carbohydrates, proteins and starch was carried out using the above extracts by following the standard methods. Alkaloids were tested by Mayer’s test and few drops of Mayer’s reagent was added to 3 ml of each extract. Dragendorff’s test was done by adding few drops of Dragendorff’s reagent to 3 ml of each extract. To 3 ml of each extract 5 ml of 95% ethanol and few drops of conc. HCl and 0.5 g magnesium turnings were added to perform flavonoids Shinoda test. Presence of carbohydrates was detected by Molish test and few drops of alpha-napthol solution in alcohol was added to 3 ml of each extract, shaken and concentrated sulphuric acid was added from sides of test tube. Test for reducing sugars was done by Benedict’s test where equal volume of Benedict’s reagent and each extract were mixed and heated in boiling water bath for 5 min. Test for cardiac glycosides was done by Legal’s test and one ml of pyridine and one ml of sodium nitropruside were added to each extract.

Test for proteins was done by Biuret test where 4% sodium hydroxide and few drops of copper sulphate were added to 3 ml of each extract and shaken well. Test for steroids was done through Salkowski test and two ml of chloroform and 2 ml of concentrated sulphuric acid were added to 2 ml of each extract and shaken well.

Test for proteins was done by Biuret test where 4% sodium hydroxide and few drops of copper sulphate were added to 3 ml of each extract. Xanthoprotein test was also done by adding one ml of concentrated sulphuric acid to 3 ml of each extract. Presence of starch was detected by Iodine test and a few drops of dilute iodine solution were added to 3 ml of each extract.
Test organisms
Bacteria causing infectious diseases both in animals and humans were used in the present study. Six Gram negative bacteria, viz. *Enterobacter aerogens*, *Klebsiella pnemoniae* (MTCC 109), *Salmonella typhi*, *Salmonella paratyphi A & B*, *Ralstonia eutropha*, six Gram positive bacteria, viz. *Staphylococcus aureus* (MTCC 96), *Bacillus cereus*, *Bacillus subtilis* (MTCC 441), *Bacillus sphericus*, *Bacillus sterothermophilus*, *Micrococcus luteus*, were used. These cultures were procured from IMTECH, Chandigarh and Kakatiya Medical College, Warangal.

Culture medium
Nutrient agar medium was used for sub culturing the pathogenic test organisms and to study the antibacterial activity of *H. arifolia*.

Inoculum preparation
Bacterial cultures were inoculated in nutrient broth tubes and incubated overnight. Inocula of these cultures were standardized using 0.5 Mc Farland solution

Assay of antibacterial activity
Antibacterial activity was done in triplicates by agar well method. Nutrient agar plates were spread with 100 µl of each inoculum by using cotton swab and 100 µl was added to 6 mm wells which were made by using sterile borer. All plates were incubated at 37°C for 18 h.

Results and Discussion
Preliminary phytochemical screening (Table 1) showed the presence of flavonoid, steroids and cardiac glycosides along with reducing sugar in all the extracts investigated, while the alkaloids, proteins and starch were absent in all the extracts. Precipitate was not formed for alkaloids (Mayer’s test and Dragendorff’s test), pink color was observed for flavonoids test (Shinoda test), violet ring was formed at the junction of two liquids for carbohydrates (Molish test), solution appeared in green colour for reducing sugars (Benedict’s test), red colour appeared for cardiac glycosides (Legal’s test), chloroform layer appeared in red color and acid layer showed greenish yellow fluorescence for steroids (Salkowski test), violet colour was not appeared (Biuret test), white precipitate was also not formed (Xanthoproteic test) for proteins and blue color was not observed for starch (Iodine test). Two dimensional paper chromatographic results revealed that FL-3-O-glycosides, FO-C-glycosides were present in *H. arifolia* leaf tissue

Table 1—Phytochemical screening results of *Hemionitis arifolia* (Burm.) Moore

<table>
<thead>
<tr>
<th>Name of the Solvent extract</th>
<th>Alkaloids Mayer’s test</th>
<th>Alkaloids Drag-en-dorff’s test</th>
<th>Flavonoids Shinoda test</th>
<th>Flavonoids Molish’s test</th>
<th>Carbohydrates Reducing sugars Benedex’s test</th>
<th>Cardiac glycosides Legal’s test</th>
<th>Steroids Salkowski’s test</th>
<th>Steroids Biuret Test</th>
<th>Proteins Xantho-protein test</th>
<th>Starch Iodine test</th>
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<td>Ethyl acetate</td>
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- = absent  + = present
outer membrane acts as a great barrier to many environmental substances including antibiotics. Presence of thick Murine layer in the cell wall prevents the entry of the inhibitors.

The present study revealed that Gram-negative bacteria such as *Salmonella typhi*, *S. paratyphi A* and *Enterobacter aerogens* were more susceptible to the crude extracts than Gram-positive bacteria. It may be due to the presence of broad spectrum of antibiotic compounds present in *H. arifolia*. Among 114 species of pteridophytes tested for their antibacterial activity, *H. arifolia* showed 0-18 mm inhibition zone on *Staphylococcus aureus*, 0-12 on *Staphylococcus aureus* (MTCC 96); *K. pneumoniae* (MTCC 109); *S. paratyphi A*; *B. cereus*; *B. subtilis* (MTCC 441); *B. sphericus*; *Klebsiella pneumoniae*; *Micrococcus luteus* and *Salmonella typhi*; *S. paratyphi B*. Some of the flavonoids that favour polar solutes entry bind to the bacteria’s structural membrane proteins called porines, causing changes in the tridimensional conformation exposing the hydrophilic character of the pore, which lead to an easier passage of other polar bioactive compounds via diffusion. Antidiabetic and hypoglycemic properties of *H. arifolia* (Burm.) Moore was carried out in rats and reported in *Staphylococcus aureus* was evaluated in vitro microtiter plate dilution methods.

**Conclusion**

The antibacterial activity of *H. arifolia* is confirmed and this can be further investigated to separate and use the biomolecules in pharmaceutical applications and development of new drugs.

**Acknowledgement**

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