Phytochemical analysis, *in-vitro* anti-microbial activity of Indian medicinal ferns *Adiantum lunulatum* and *Hemionitis arifolia*

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**ABSTRACT**

**Background:** The aim of this study is to determine the phytochemical analysis and antimicrobial activities of aqueous, ethanol and petroleum ether extracts of *Adiantum lunulatum* and *Hemionitis arifolia* against *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 432), *Proteus mirabilis* (MTCC 425), *Salmonella paratyphi* A (MTCC 735), *Salmonella paratyphi* B (Clinical isolate), *Salmonella typhimurium* (MTCC 98), *Shigella dysenteriae* (Clinical isolate), *Pseudomonas aeruginosa* (MTCC 424), *Vibrio parahaemolyticus* (MTCC 451), two fungus, *Candida albicans* (MTCC 183) and *Cryptococcus neoformans* (Clinical isolate). **Materials and Methods:** Antimicrobial activity was evaluated by the disc diffusion method and phytochemical constituents were identified by the standard procedures. **Results and Discussion:** The aqueous extracts of *A. lunulatum* showed significant inhibitory activity against *C. neoformans*, *S. paratyphi* B, *P. aeruginosa* and *V. parahaemolyticus*. Ethanol extract showed inhibitory activity against *P. aeruginosa*, *S. epidermidis* and *S. paratyphi* B. The petroleum ether extract showed significant inhibitory activity against *S. flexneri*, *S. typhimurium*, *C. albicans* and *K. pneumoniae*. The aqueous extract of *H. arifolia* showed inhibitory activity against *P. aeruginosa*, *S. aureus* and *S. paratyphi* A. Ethanol extract showed better inhibitory activity against *P. aeruginosa*, *C. neoformans*, *S. paratyphi* A, *Vibrio parahaemolyticus* and *S. aureus*. The petroleum ether extract showed maximum inhibitory activity against *P. aeruginosa*, *S. aureus*, *E. coli* and *S. epidermidis*. **Conclusion:** This study concludes that the antimicrobial properties of *A. lunulatum* and *H. arifolia* might be associated with the presence of minor and major phytoconstituents.

**KEY WORDS:** *Adiantum lunulatum*, *Hemionitis arifolia*, Phytoconstituents, Antimicrobial activity.

**INTRODUCTION:** Medicinal plants have been used for centuries as remedies for human diseases and other organisms because they contain certain components of therapeutic value. Medicinal plants are a source of great economic value all over the World. Natural products from the plants may become a new richest resource of drugs of traditional system of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The medicinal value of plants lies in thousands of chemical substances that produce a definite physiological action on the human body, which are most important of these bioactive compounds of plants are, flavonoids, tannins and phenolic compounds that could be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Medicinal plants are an important source of inexpensive and practical drugs for people throughout the world. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. As a result, recently some higher plant products have attracted the interest of
microbiologist and pharmacologist to search for phytochemical for their use as antimicrobials. Such plant products would be biodegradable and safe to human health.  

Adiantum lunulatum (Burm.) or Phillippense (Linn.) belongs to the family Adiantaceae. It is a leafy fern, stipes dark, chestnut-brown, wiry, 6-15 cm long, glabrous, shining tufted. Fronds often elongated, rooting at apex, pinnate, pinnae sub-dimidate, sori borne in a continuous line along the edge. The whole plant are traditionally used for treating asthma, cough, bone fracture, pimples, atrophy, cachexy, emaciation, watery diarrhea and fever. Leaf are used for curing balding, erysipelas, leprosy, bronchitis and spray and roots are used for fever, muscular pain, rabies, bite of a rabid jackal or dog. In Ayurveda plants are used for anti-dysenteric, febrifugal, subdues burning sensation of body, useful as soothing agent (as lotion) in erysipelas and along with Asparagus racemosus in gonorrhea. Currently it is used in blood diseases and epileptic fits. Rhizome is prescribed for strangury and fever due to elephantiasis. Fronts are burnt in oil and applied to itch. Chemical Constituents- plant is higher carotenoids and chlorophyll-degradation products.

Hemionitis arifolia (H. arifolia) (Burm.) Moore (family: Hemionitidaceae) is a small, terrestrial, rhizomatous fern, rhizomes short, 5-10 cm long, sub-erect, covered dimorphic, sterile ones deeply cordate, fertile ones sagitate, stiped, shining green, coriaceous, reticulately veined, sori covering the entire lower surface. Identified to science in 1859, H. arifolia is an attractive and unusual dwarf fern primarily from Laos, Sri Lanka, Vietnam and possibly China, Taiwan and other nations in tropical Southeast Asia. The fern is both an epiphyte and grows on trees as well as a terrestrial plant. It is also used in folk medicinal practices to treat diabetes mellitus in certain remote villages of Trivandrum district, Kerala state. This fern is also used to treat aches and burns. Leaf extract is applied to centipede bite and wounds and about 10g of root powder is taken orally with water in empty stomach twice a day for 10 days for treatment of hypertension. Plants are found to be an immense source for variety of bioactive molecules with diverse molecular structure and function.

In recent years, pharmaceutical companies have spent substantial time and money in developing therapeutics based upon natural products extracted from plants. In the present approach involved to explore the antimicrobial activity and phytochemical analysis of A. lunulatum, H. arifolia.

MATERIALS AND METHODS:

a) Collection of plant materials:
The whole plants of A. lunulatum and H. arifolia were collected from the natural habitat of Ponmudi hills, Kerala, India. The plant was primarily identified and confirmed by the scientists Dr. Santhosh kumar, Scientist of Tropical Botanic Garden and Research Institute, Thiruvananthapuram, Kerala, India.

b) Extraction of Plant Materials:
The plant materials were shade dried and pulverized. About 250g of powdered material was packed in Soxhlet apparatus and subjected to continuous hot percolation for 8h using 450ml of petroleum ether, aqueous and ethanol as solvent. All the extracts were concentrated under vacuum and dried in a dessicator.

c) Phytochemical Analysis:
Various extracts of A. lunulatum, H. arifolia were analyzed for the phytochemical constituents viz., carbohydrates, glycosides, fixed oils and fats, proteins and amino acids, saponins, tannins, phytosterol, alkaloids, phenolic compounds, flavonoids and gums and mucilages etc., using standard procedures described.

d) Antimicrobial screening

a. Source of microbial strains
A total of 14 human pathogenic microorganisms used in this study were S. aureus (MTCC 96), E. coli (MTCC 739), K. pneumoniae (MTCC 432), P. mirabilis (MTCC 425), S. paratyphi A (MTCC 735), S. paratyphi B (Clinical isolate), S. typhimurium (MTCC 98), S. dysentriae (Clinical isolate), P. aeruginosa (MTCC 424), V. parahaemolyticus (MTCC 451), two fungus, C. albicans (MTCC 183) and C. neoformans (Clinical isolate). The microorganisms were originally obtained from Microbial Type Culture Collection Centre (MTCC), Institute of Microbial Technology, Chandigarh, India. Clinical isolate was collected from local clinical laboratory at Namakkal.

b. Determination of antimicrobial activity:

Minimum Inhibitory Concentration (MIC) by Disc Diffusion method
The antimicrobial activities of A. lunulatum, H. arifolia (Aqueous, Ethanol, Petroleum ether extract) were tested by disc diffusion method. The culture plates were prepared by pouring 20ml of sterile Hi-sensitivity (Himedia- M 486) agar medium. The depth of the medium was approximately 4mm. Three to four similar colonies of pure cultures were inoculated with tryptone soy broth (Himedia- M 323), further, it was incubated at 37°C for 2-8h and inoculum size was adjusted to yield uniform suspension containing 10^5-10^6 cells/ml (McFarland’s standard). The agar surface of the plates was swabbed in three directions, turning the plates at 60° between each swabbing.
Confluent growth is desirable for accurate results. The sterile discs (6 mm- Himedia) were used for the loading plant extracts. Five different concentrations were prepared (100, 200, 300, 400, and 500µg) and loaded in appropriate discs. The impregnated discs were incubated at 37°C for 1 h. The dried discs were placed over the surface of swabbed medium with equal distance to avoid the overlapping of the inhibition zone. Then discs were pressed gently on the surface of the medium. Allowed the plates to stand at refrigerator for 30min (Pre-diffusion time). The plates were incubated at 37°C for 16-18h during which the activity was evidenced by the presence of zones of inhibition surrounding the discs. Each experiment was done in triplicate. A panel of antibiotics was used against each microbial strain and which antibi-otic given sensitive with particular organism is used as control.

c. Statistical analysis:
In this study, Inhibitory activity of different concentrations of plant extracts was expressed in mean and standard error of mean.

### Results:

#### Phytochemical Analysis:
The preliminary phytochemical analysis results of *A. lunulatum*, and *H. arifolia* (aqueous, ethanol and petroleum ether extracts) were recorded (Table 1). *A. lunulatum* extracts contains phenols, saponins, tannins, xanthoproteins, carboxylic acids, carbohydrates and *H. arifolia* extracts contains phenols, cardiac glycosides, saponins, flavonoids, steroids, coumarin, xanthoproteins, tannins, carbohydrates.

#### Minimum Inhibitory Concentration (MIC) by Disc Diffusion method:
The results of anti-bacterial and anti-fungal activities of crude extracts of *A. lunulatum* were screened by disc diffusion method against 14 microorganisms including 2 fungi and the mean values of zone of inhibition were recorded (Table 2). Aqueous extract showed

### Table 1: Phytochemical (qualitative) analysis of *Adiantum lunulatum* and *Hemionitis arifolia* (aqueous, ethanol, petroleum ether extracts)

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th><em>Adiantum lunulatum</em></th>
<th><em>Hemionitis arifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extract</td>
<td>Ethanol extract</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Xanthoproteins</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2: Antimicrobial activities of *Adiantum lunulatum* and *Hemionitis arifolia* (aqueous, ethanol, petroleum ether extracts):

<table>
<thead>
<tr>
<th>Name of the Microorganisms</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Adiantum lunulatum</em></td>
</tr>
<tr>
<td></td>
<td>Aqueous extract Mean ± SEM</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>-</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. paratyphi</em> A</td>
<td>-</td>
</tr>
<tr>
<td><em>S. paratyphi</em> B</td>
<td>8.00 ± 1.00</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>-</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>8.60 ± 0.60</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>6.80 ± 2.80</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>-</td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>10.00 ± 1.09</td>
</tr>
</tbody>
</table>

SEM= Standard Error of Mean
The antimicrobial activity of *H. arifolia* (aqueous, ethanol, petroleum ether extracts) were screened by disk diffusion method against 14 microorganisms including 2 fungi and the mean value of zone of inhibition was given in Table 2. The aqueous extract showed significant inhibitory activity against *P. aeruginosa* (23.60mm), and slight activity with *S. aureus* (8.40mm), *S. paratyphi B* (8.00mm), and *V. parahaemolyticus* (7.00mm). Ethanolic extract showed significant inhibitory activity against *P. aeruginosa* (23.00mm), moderate activity with *C. neoformans* (18.40mm), slight activity with *S. paratyphi A* (9.20mm), *V. parahaemolyticus* (8.60mm) and *S. aureus* (8.40mm). The petroleum extract showed moderate inhibitory activity against *S. epidermidis* (8.60mm), *B. subtilis* (8.40mm) and *C. neoformans* (7.40mm).

**DISCUSSION:**
The development of resistance in microorganisms to antibiotics and emergence of new infectious diseases create urgent need to discover novel, safe and effective antimicrobial compounds. The improper usage of antibiotics contributes a major role for drug resistance in pathogenic microbes. Microorganisms acquire resistance towards common antibiotics by altering their metabolism and genetic structure. This situation has forced scientists to search for new antimicrobial substances in various sources like medicinal plants. Therefore, medicinal plants which possessing antimicrobial property is getting attention today. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. The current study was initiated because of the increasing resistance to antibiotics of many pathogens including bacteria and fungi.

The present investigation was an attempt to explore the antimicrobial activity and qualitative phytochemical analysis of *A. lunulatum*, *H. arifolia*. Compounds derived from *A. lunulatum* have been shown antibacterial activity against gram positive and negative bacteria particularly *S. typhi* and *P. aeruginosa*. Similarly in the present work, aqueous extract showed maximum inhibitory activity against most of the gram negative bacteria and fungi viz., *V. parahaemolyticus*, *P. aeruginosa*, *C. neoformans*, *S. paratyphi B*. The alcohol and aqueous extracts of *A. lunulatum* were effective against *E. coli*, *S. typhi* and *S. aureus*. The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds. Likewise in the present study aqueous extracts of *A. lunulatum* contains the phenol, tannins, xanthoprotein and carbohydrate, in ethanolic extract phenol, carboxylic acid and carbohydrate, whereas in petroleum ether phenol and saponins. Similar phytochemical results of *A. lunulatum* were also reported. *A. lunulatum* exhibits strong bioactivities, especially analgesic, antinociceptive, antiimplantation, and antimicrobial activities. *A. lunulatum* contains Isohpane-type triterpenoids, 10 and Hopane-type triterpenoids, 1-9. *A. philippense* Linn (*A. lunulatum* Burm. f.) also possess anti-hyperglycemic as well as antioxidant potential.
A. lunulatum and H. arifolia is confirmed and this can be further investigated to separate and used in the pharmaceutical application for the development of new drugs.

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CONFLICT OF INTEREST STATEMENT:
We declare that we have no conflict of interest.

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