

PHYTOCHEMICAL SCREENING AND ITS ANTIBACTERIAL ACTIVITY OF THE FERN *HEMIONITIS ARIFOLIA* (BURM.) MOORE

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ABSTRACT

The aim of the present study was to investigate the phytochemistry and antibacterial activity of the fern *Hemionitis arifolia* (Burm.) Moore. The methanol leaf extracts of this fern was investigated for qualitative phytochemistry and antibacterial analysis. The antibacterial activity was studied against different bacterial strains viz. *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus sp* and *Klebsiella pneumoniae* by agar well method. The zone of inhibition was compared with standard antibiotic of Ampicilin. The highest antibacterial activity was observed against gram positive bacteria *Enterococcus faecalis* at 100 mg/ml concentration. The qualitative

analysis revealed the presence of phenols, saponins, tannins, steroids, flavonoids, glycosides and carbohydrates.

KEYWORDS: *Hemionitis arifolia*, Phytochemical analysis, antibacterial activity, well diffusion method.

INTRODUCTION

Pteridophytes (ferns and fern allies) are called as reptile group of plants and are one of the primary groups of vascular plants. Most of the indigenous people are not well identified about the uses of Pteridophytes ever since it's not simply available like flowering plants. Pteridophytes are playing a major role in biodiversity. In the world flora of pteridophytes 12,000 species has been identified among which 1,000 species (70 families and 191 genera) are present in India (Dixit, 1975).

The ferns had an important role in folklore medicine. Pteridophytes have been used in the Ayurvedic, Unani, Homeopathic and other systems of medicines. The pteridophytes are

mostly distributed in the Himalayas. More than 300 species of ferns and fern allies are reported from the Western Ghats, South India (Manickam and Irudhayaraj 1992).

Pteridophytes are not infected by microbial pathogens, which may be one of the significant aspects for the evolutionary achievement of pteridophytes and the information that they stay alive for more than 350 million years. In view of the rich diversity of Indian medicinal plants as well as Pteridophytes, it is predictable that, the screening of plant extract for antibacterial activity might be helpful for humans and plants diseases (Sharma and Vyas, 1985). In recent years antibiotic resistance has become a global concern. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects (Prusti *et al.*, 2008).



Figure. 1. *Hemionitis arifolia* (Burm.) Moore.

Hemionitis arifolia (Burm.) Moore of family *Hemionitidaceae* is one of the endemic and widely distributed species on Tirumala hills of Tirupathi, Andhra Pradesh, India. Ethno medicinally, the genus is important and popularly known as Ramabanum. This fern presents itself with dark green heart-shaped fronds, about 2-3 inches long and borne on black stems, and reaches a height of between 6-8 inches tall. Leaves are dimorphic, meaning some are sterile and some are fertile. The sterile fronds are heart shaped upon a 2- to 4-inch thick stalk, while the fertile fronds are shaped like an arrowhead on a thicker stalk. The fronds are not the stereotypical fern leaves. Heart fern's foliage is thick, leathery and slightly waxy. *Hemionitis arifolia* has been used in burns, menstrual disorders, antifertility and anti-flatulence. The leaf juice has been used to cure burns and diabetes (Mannar *et al.*, 2008). Though the recent ethno botanical, pharmacological and biological searches have revolved 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. There are no reports on the phytochemical and antibacterial activity of the fern *Hemionitis arifolia* (Burm.) Moore. Hence, the aim of the study was investigate the phytochemical and antibacterial activity of the fern *Hemionitis arifolia* (Burm.) Moore.

MATERIALS AND METHODS

Selection and collection of plant material

Hemionitis arifolia (Burm.) Moore was collected from Western Ghats of Tamilnadu, South India and the fern was identified by the PG and Research Department of Botany, NGM College, Pollachi.

Phytochemical Analysis

The leaf of *Hemionitis arifolia* (Burm.) Moore was shade dried and Powdered. The methanol extract was prepared according to the method described by Harbone, 1998. The sample was sequentially extracted using a Soxhlet apparatus and was subjected to detect the presence of bioactive compounds according to the method described by Kokate, 1994; Harborne, 1998; Krishnamurthy, 1988; Salkowski and Balish, 1991; Brain and Turner, 1975; Ciulci, 1994 and Gokhal *et al.*, (2008).

Antimicrobial activity

Preparation of crude extract

Dried methanol extract 100 mg was dissolved in 1ml of DMSO solvent. From this stock different concentration (12.5-100 mg/ml) of extracts were prepared. Antibacterial activity of the extract was assayed separately using agar well diffusion method (Azoro, 2002) against pathogenic bacteria such as *Enterococcus faecalis*, *E.coli*, *Klebsiella pneumoniae*, *Bacillus sp* and *Pseudomonas aeruginosa*. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 12 hours-old broth culture of respective bacteria. Using the sterile cork borer, the well (8 mm) was made into the each Petri-plate. 100 µl of different concentration (12.5-100 mg/ml) of methanol extracts of *in vivo* leaves of *Hemionitis arifolia* was added to the well separately. Then the plates were incubated at 37°C for 16-24 hours. The antibacterial activity of the extract was assessed based on the measurement of the inhibition zone formed around the well. Ampicillin was served as positive controls and the DMSO was used as negative control to compare the activity of the extracts.

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical screening of methanolic extract of *Hemionitis arifolia* showed the presence of phenols, saponins, tannins, steroids, flavonoids, glycosides and carbohydrates. Similar result was observed in *Hemionitis sp.*, which contains phenol, saponins, Terpenoid, tannin, flavonoids, sterols, glycosides. Similar results was also observed by Varaprasadham *et al.* (2010) in the methanol extract of *Selaginella inaequalifolia* (hook. & grev.) for the presence of bioactive compounds. The results showed the presence of steroids, tannin, triteropenoids and saponins. Muhammad *et al.*, (2014) in methanol extract of *Adiantum capillus veneris* is also showed the similar result. Similarly, Herin *et al.*, (2013) in methanol extract of *Pteris argyreae*, *P.confuse*, *P.vittata*, *P.biaurita* and *P.multiaurita* were showed phytochemicals like steroids, alkaloids, saponins, flavonoids, triteropenoids, phenolic compounds and tannins were also found in *Hemionitis arifolia*.

Antibacterial activity of *Hemionitis arifolia* leaf extracts

The methanol extract of *in vivo* frond of *Hemionitis arifolia* was analysed for antibacterial activity against *Enterococcus faecalis*, *E.coli*, *P.aeruginosa*, *Bacillus sp* and *K.pneumonia*. The results were compared with the standard broad spectrum antibiotic ampicillin, which was used as positive control and the DMSO served as negative control. The results were represented in table (Table- 1). Zone of varying diameter were obtained with various organisms in different concentrations of extract tested. The maximum zone of inhibition was observed in *Enterococcus faecalis* (21 mm) followed by *Bacillus sp.*(20 mm), *E.col* (19mm) *K.pneumoniae* (19 mm) and *P.aeruginosa* (19 mm) at 100 mg/ml concentration. The minimum zone of inhibition was observed in *P.aeruginosa* (12mm) at 12.5 mg/ml concentration.

Saleha *et al.* 2014 studied the antibacterial activity in *Diplazium esculentum* (Retz). The antibacterial activity was determined by disc diffusion method. The highest zone of inhibition in observed for *Sarcina lutea* (18.67mm) followed by *Salmonella typhimurium* (16.33mm). The extracts showed relatively lower antibacterial activity against *Klebsella pneumoniae* (15.33mm) followed by *Bacillus* (15.33mm), *E.coli* (12.33mm) at 300µg/disc. In the present study highest zone of inhibition was observed in *Klebsiella pneumonia*, *Bacillus sp* and *E.coli* at 12.5 mg/ml concentration.

Table. 1. Antibacterial activity of *Hemionitis arifolia* (Methanol extract).

Organisms	Zone of inhibition (mm)					
	Concentration (mg/ml)				Ampicillin 10µl (50µg/ml)	DMSO
	12.5	25	50	100		
<i>E.coli</i>	13	15	16	19	23	0
<i>K.pneumoniae</i>	13	15	17	19	23	0
<i>P.aeruginosa</i>	12	14	17	19	23	0
<i>Bacillus sp.</i>	14	15	17	20	30	9
<i>Enterococcus faecalis</i>	16	18	20	21	17	0

Methanolic leaf extract of *Lygodium flexuosum* was showed the zone of inhibition in *K. pneumonia* (22 mm), *P. aeruginosa* (19 mm) and *E.coli* (14 mm) (Naba kishore *et al.*, 2013). Similar results were also observed in *Hemionitis arifolia*. Bahadori *et al.* 2015 showed the antibacterial activity of some ferns. The maximum activity was exhibited by the extract of *Dryopteris affinis* with MIC value of 2 µl/ml. *Polystichum aculeatum* showed the same antibacterial potential against *S. aureus*. In methanolic extract of *Hemionitis arifolia* showed maximum antibacterial potential against *Enterococcus faecalis*.

Antibacterial properties of frond-extracts using the solvent mixture, methanol: dichloromethane at 1:1 with 5 fern species, *L. flexuosum*, *Selaginella bryopteris*, *Adiantum philippense*, *Dryopteris eochleata* and *Tectaria coadunata* had been recorded against human pathogen *P. aeruginosa* (Malviya *et al.*, 2012). Similar result was observed in methanol extract of *Hemionitis arifolia* which inhibit *P. aeruginosa*. Antimicrobial activity of crude extracts of the epiphytic fern, *Arthromeris himalayensis* had been recorded against *B. subtilis* and *E. coli* by the agar well diffusion method (Ganguly *et al.*, 2011). Similar results were observed in the in methanol extract of *Hemionitis arifolia*.

CONCLUSION

Based on our study, it is concluded that the fern *Hemionitis arifolia* methanol fraction have significant antibacterial activity along with valuable secondary metabolites. Further studies are recommended to find the antifungal and antiviral activity of *Hemionitis arifolia* extract against pathogenic organisms.

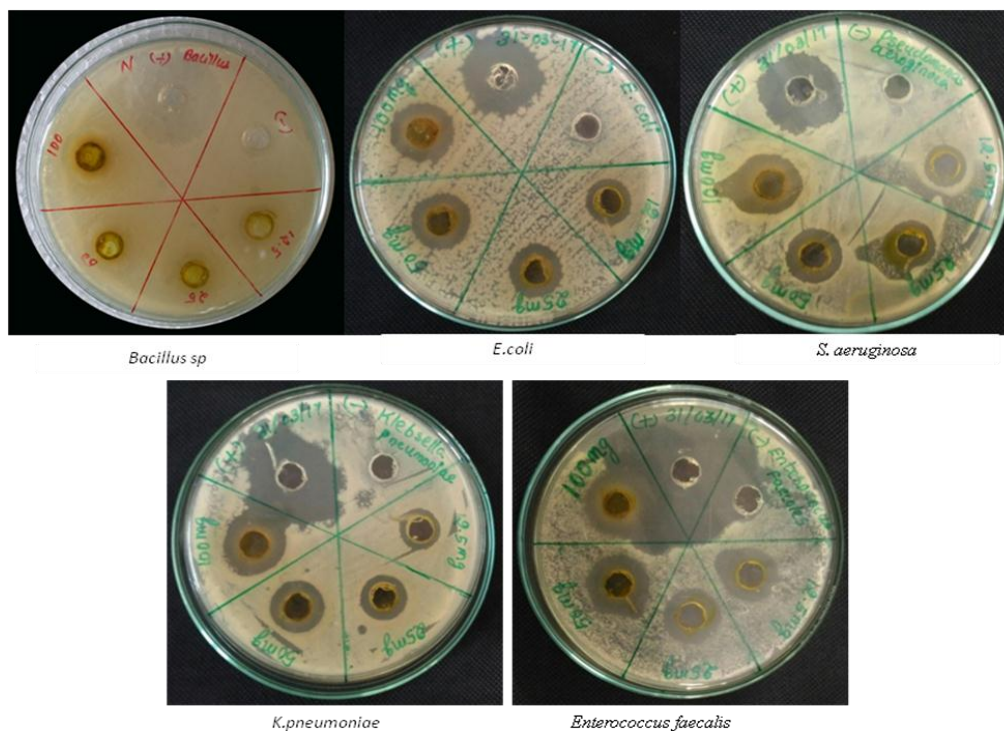


Figure. 2: Antibacterial activity of methanolic extract of *Hemionitis arifolia*.

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