

**PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTY OF  
*GLAPHYROPTERIDOPSIS ERUBESCENS* (HOOK.) CHING,  
WESTERN GHATS, SOUTH INDIA**

\*John Peter Arulanandam .L<sup>1</sup> and John De Britto A<sup>2</sup>

<sup>1</sup>Centre for Biodiversity and Biotechnology,

<sup>2</sup>Plant Molecular Biology Research Unit

St. Xavier's College, Palayamkottai – 627 002

\*Email: [jparulsj@gmail.com](mailto:jparulsj@gmail.com), Tel & Fax : 0462-2561987

### Summary

The preliminary phytochemical study on the hexane, ethyl acetate and methanol extracts of *Glaphyopteridopsis erubescens* revealed the presence of active phytochemical constituents such as steroids, terpenoids, saponins, flavonoids, tannins, reducing sugar, phenolic compounds and coumarins. Like wise, when these extracts were subjected to antimicrobial study, it was found that the growth of the gram positive bacterium *Staphylococcus aureus* and *Enterococcus faecalis* were inhibited respectively.

**Key words:** Phytochemical, Antimicrobial, *Glaphyopteridopsis erubescens*, Phenolic compounds

### Introduction

The World Health Organization (WHO) has estimated that up to 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant materials [1]. Higher plants, as sources of medicinal compounds continue to play a dominant role in the maintenance of human health since antiquities. Like angiosperms, pteridophytes also show medicinal utility and many of them like male fern or shield fern are being used medicinally from the time of Theophrastus and Dioscorides [2]. In the present study, the fern species *Glaphyopteridopsis erubescens* was subjected to preliminary phytochemical as well as antimicrobial studies to observe their bioefficacy and establish their medicinal usefulness.

### Materials and Methods

#### a) Preliminary phytochemical analysis

The shade dried fronds with spores of *G. erubescens* were powdered by using an electric blender. The powdered plant materials were stored in tightly closed separate glass containers at room temperature. To determine the efficacy of different extractants, the

powdered frond materials were extracted in three technical grade solvents of varying polarity namely hexane, ethyl acetate and methanol. To begin with, 100 gms each of dried fronds powder of this plant was immersed in 500 ml of hexane in separate 1000 ml conical flasks and allowed to remain for 72 hours at room temperature and the contents was shaken occasionally. Then the extracts were filtered with the help of Whatman No.1 filter paper and the solvent from the filtrates was evaporated using Vacuum Rotary Evaporator at 40°C. From this, the crude extracts were collected in sterile screw capped bottles, labeled and stored at -20°C.

The remaining plant residue was extracted with ethyl acetate and methanol sequentially. After the extraction with each solvent, the plant materials were air dried at room temperature for complete evaporation of extracting solvent before subsequent extraction. The quantity of the solvents, the time duration of plant materials' immersion in the solvents and the rest of the procedure followed for obtaining the crude extracts of these two solvents were the same as it was done for hexane extraction.

The crude extracts of the fern *G. erubescens* extracted in three different solvents namely hexane, ethyl acetate and methanol were taken in the test tubes and diluted to a known volume by adding the respective solvents and were subjected to preliminary phytochemical analysis [3]. They were analysed for the presence of alkaloids, flavonoids, terpenoids, steroids, tannins, saponin, reducing sugar, quinones, phenolic compounds, sugar and coumarins.

#### **b) Antimicrobial studies**

The same hexane, ethyl acetate and methanol plant extracts were also used to evaluate the antimicrobial properties by Disc diffusion method [4] against ten disease causing pathogens namely *Xanthomonas oryzae*, *Salmonella typhi*-B, *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (MTCC 227), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (MTCC 441), *Erwinia amylovora* (MTCC 2760), *Proteus vulgaris* (MTCC 1771), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 15380) that were procured from American Type Culture Collection (ATCC), USA and Microbial Type Culture Collection (MTCC), Chandigarh respectively.

The growth media required for the study were prepared by using Mueller Hinton Agar (MHA) High media, India. The plant extracts were reconstituted in 10% Dimethylsulfoxide (DMSO) and diluted with sterile distilled water to a concentration of 200 mg / ml. The sterile filter paper discs (6 mm in diameter) were then individually impregnated with 25 µl volume containing 5 mg concentration of plant extracts. The loaded discs were allowed to dry for sometime and then they were placed on the surface of the medium. Standard streptomycin discs (10 µg/disc) were used as positive control. Negative control was prepared by impregnating sterile filter paper discs with a solution that contains 10% DMSO and sterile distilled water. The plates inoculated with bacteria were incubated at 37°C for 24 hours and at 30°C for 48 hour for the fungus. The assessment of bacterial and fungal activity was based on the measurement of diameter of zones of inhibition formed around the discs. The petriplates were labeled appropriately. Triplicates of each plate were placed.

**Results and Discussion**

**a) Preliminary phytochemical study**

The secondary metabolites like alkaloids, steroids, phenolics, flavonoids may be produced as defense mechanisms by the plant and also they have a wide range of applications in the pharmaceuticals, chemical and food industries [5]. In the present preliminary phytochemical screening of the plant *G. erubescens*, the Hexane extract revealed the presence of only steroids. Ethyl acetate extract showed the presence of terpenoids, steroids and saponins. Methanol extract showed positive results for the presence of flavanoids, terpenoids, tannin, saponin, reducing sugar, phenolic compounds and coumarin. Among these, the presence of saponin and phenolic compounds are reported to be more intense than any other compound.

Although ferns have been used for medicinal purposes for a long time, they have not been adequately screened for phytochemical properties when compared to the flowering plants. However some reports are available on the phytochemical screening of ferns. The analysis on *Cyclosorus interruptus* (Willd.) revealed the presence of three new bioactive coumarin derivatives [6]. The results of the present preliminary phytochemical investigation are almost similar to the earlier reports[7,8,9] who have observed the presence of coumarin, steroid, alkaloid, phenol, catechin, saponin and tannin and flavanoids in the nineteen species of South Indian Thelypteroid ferns. Preliminary phytochemical screening of the family pteridaceae and the results showed that all the fern taxa possessed steroids, sugars, alkaloids, phenols, flavonoids, saponins, tannins and amino acids [10] which are in agreement with the present results. A new triterpenoid from the fern *Adiantum lunulatum* has been isolated when it was subjected to phytochemical analysis [11].

**Table 01: Preliminary phytochemical screening results of *Glaphyopteridopsis erubescens***

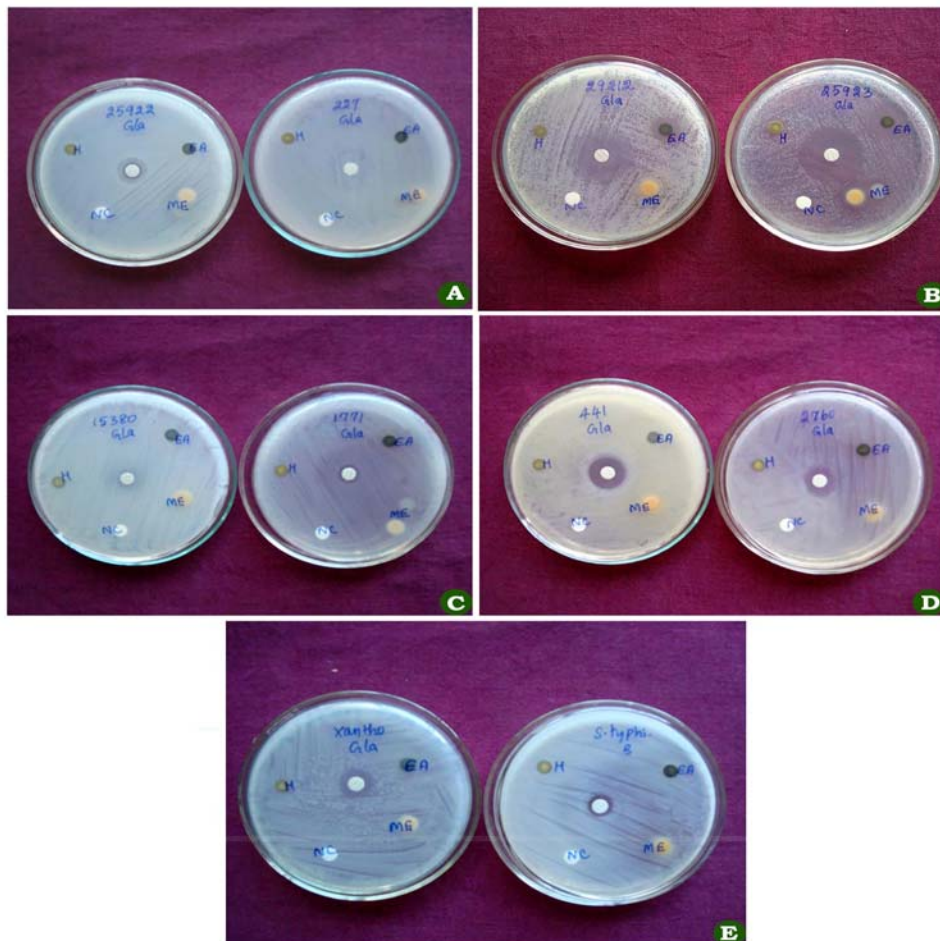
S. No	Class components	Hexane	E. Acetate	Methanol
01.	Alkaloids	-	-	-
02.	Flavonoids	-	-	+
03.	Terpenoids	-	+	+
04.	Steroids	+	+	-
05.	Tannins	-	-	+
06.	Saponins	-	+	++
07.	Reducing sugars	-	-	+
08.	Quinone	-	-	-
09.	Phenolic compounds	-	-	++
10.	Sugar	-	-	-
11.	Coumarins	-	-	+

- Absence of compounds
- + Presence of compounds
- ++ Presence of compounds more intense

## b) Antimicrobial study

In the present study, *G.erubescens* was subjected to antimicrobial study to find their bio efficacy against the growth of the pathogens which showed some encouraging results. Among the pathogens tested, the growth of the gram positive bacterium *Staphylococcus aureus* was inhibited in the methanol extract with 20 mm inhibition zone followed by ethyl acetate extract with 18 mm inhibition zone whereas hexane extract showed no activity against the same pathogen (Plate 01B).

## Plate - 1

Antimicrobial activity of *Glaphyopteridopsis erubescens*

- A - Antimicrobial activity against *Escherichia coli* and *Candida albicans*.  
 B - Antimicrobial activity against *Enterococcus faecalis* and *Staphylococcus aureus*  
 C - Antimicrobial activity against *Klebsiella pneumoniae* and *Proteus vulgaris*.  
 D - Antimicrobial activity against *Bacillus subtilis* and *Erwinia amylovora*.  
 E - Antimicrobial activity against *Xanthomonas oryzae* and *Solmonella typhi-B*

The ethyl acetate extract showed significant activity against *Enterococcus faecalis* with 14 mm inhibition zone but the hexane and methanol extracts of *G. erubescens* did not have any activity against the same pathogen (Plate 01B; Table 02).

**Table 02: Antimicrobial activity of *Glaphyopteridopsis erubescens*  
Zone of inhibition indicated in (mm) (5mg/disc)**

S.No	Organism	Hexane	Ethyl acetate	Methanol	Positive control
01.	ATCC 25922	-	-	-	07
02.	ATCC 25923	-	18	20	28
03.	ATCC 29212	-	14	-	20
04.	ATCC 15380	-	-	-	R
05.	MTCC 00441	-	-	-	15
06.	MTCC 02760	-	-	-	12
07.	MTCC 01771	-	-	-	08
08.	MTCC 00227	-	-	-	R
09.	<i>Xanthomonas oryzae</i>	-	-	-	12
10.	<i>Solmonell typhi-B</i>	-	-	-	10

- No activity :ATCC (American Type Culture Collection, USA)
- Acetone as negative control :MTCC (Microbial Type Culture Collection, Chandigarh)
- Streptomycin was used as Positive control ; R- Resistant

The phenolic compounds possessed anti-microbial and antifungal effect [12]. It was observed from the antimicrobial studies that the growth of gram positive bacteria namely *Staphylococcus aureus* and *Enterococcus faecalis* were inhibited significantly. Earlier antimicrobial studies on gametophytes as well as sporophytes have been carried out [13,14]. However in the present study, only sporophytes (fronds with spores) were used so as to get preliminary information on the antimicrobial nature of these plants.

It is evident from the results obtained in the present study, that gram positive bacterium *S. aureus* was the most susceptible bacterium studied which is similar to the reports obtained [15] who observed that out of the 114 species of Pteridophytes studied, 20 of them were inhibitory to penicillin-resistant *Staphylococcus aureus*. It was reported that the methanol extract of *Drynaria quercifolia* showed zone of inhibition on agar medium of cultures like *staphylococcus aureus*, and some other bacterial organisms [16].

It was observed that only ethyl acetate extract inhibited the growth of all the three pathogens namely *S. aureus*, and *E. faecalis* suggesting that it is the appropriate solvent for extraction of the antimicrobial properties of this plant followed by methanol and hexane extracts which are in conformity with the reports [17]. It was reported that ethyl acetate extract exhibited the highest antimicrobial activity while the n-hexane fraction exhibited the least antimicrobial activities. From these two studies certain concrete data have been obtained to say that this fern species has to be conserved as it possesses medicinal properties that are useful for the well being of the humanity.

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