Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(2):1167-1172

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Anti-bacterial efficacy of macroscopic, microscopic parts of sporophyte and *in vitro* cultured gametophyte of a fern *Cyclosorus interruptus* (Willd.) H. Ito (Thelypteridaceae - Pteridophyta)

Pauline Vincent C, Irudayaraj V, Johnson M*

Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India

---

ABSTRACT

Antibacterial efficacy of acetone extract, from different parts of sporophyte of the medicinal fern Cyclosorus interruptus (Willd.) H. Ito (Thelypteridaceae, Pteridophyta), was studied by disc diffusion method by using Staphylococcus aureus as test organism. Sporophytic parts such as rhizome, roots, rachis, sporangia and spores showed nil activity. The in vitro cultured gametophytes also showed nil activity. Only leafy parts of the sporophyte such as, croziers (young leaves), sterile and fertile mature leaves and rachis with leaves showed antibacterial activity with the inhibition zone from 18 to 28mm. The microscopical epidermal glands on leaves showed maximum antibacterial efficacy. The crude acetone extract and the ethyl acetate fraction of the crude extract of the epidermal glands showed comparatively higher degree of antibacterial efficacy followed by chloroform fraction of crude extract. All the six concentrations (5, 100, 250, 500, 750, 100µg) of chloroform fraction of crude extract showed antibacterial efficacy with the inhibition zone from 8-11mm. But the ethyl acetate fraction showed maximum size of inhibition zone 13mm in 100 µg. The acetone extracts of Cyclosorus interruptus revealed the highest antbacterial activity at a minimum concentration 31.255µg/100µl against S. aureus. Among the macroscopical and microscopical parts of sporophyte of the medicinal fern Cyclosorus interruptus, the leafy part of the sporophyte show maximum antibacterial activity for which the epidermal glands with various bioactive compounds play a major role.

Key words: *Cyclosorus interruptus*, sporophyte, gametophyte, epidermal glands, antibacterial efficacy.

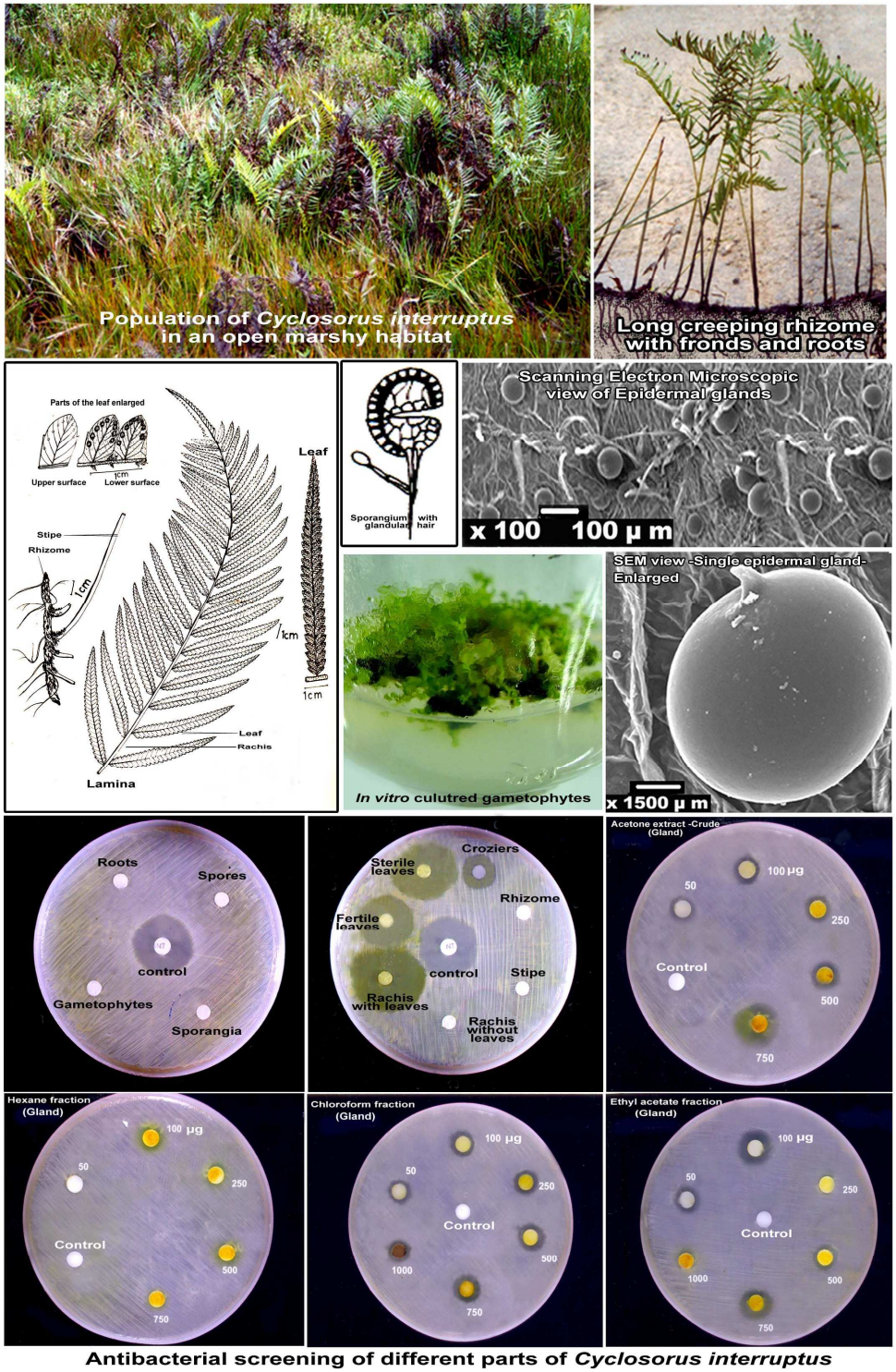
INTRODUCTION

Plants are the important source of several modern drugs which have been developed based on the valuable ethnomedicinal information on medicinal plants. India is a megabiodiversity country not only with rich source of medicinal plants, but also with valuable information on traditional medical practices. Fabricant and Farnsworth [1] described and discussed several approaches to select higher plants as candidates for drug development with the greatest possibility of success. They have emphasized the role of information derived from various systems of traditional medicine (ethnomedicine) and its utility for drug discovery purposes. They have identified 122 compounds of defined structure, obtained from only 94 species of plants that are used globally as drugs and demonstrate that 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant. In general, majority of drug development processes include screening of macroscopical parts such as aerial parts stem or leaf or both, underground part, roots and reproductive part like seeds, rarely flowers [2].

In the modern nanotechnological world, nanoparticles have emerged as important players in modern medicine, with clinical applications ranging from contrast agents in imaging to carriers for drug and gene delivery into tumors [3]. It is understood that when compared to macroscopical parts / particles, the microscopical parts / particles and nanoparticles are more effective. In plants also, there are several microscopical parts such as epidermal glands, hairs etc. which play an important role in the survival of the plant with the presence of variety of chemical compounds. But screening for valuable compounds present in such microscopical parts of the plants is very rare. Recently, Paul Raj *et al.* [3] have studied the phytochemistry and antibacterial activity of microscopical epidermal glands of a fern *Christella parasitica* (L.) H. Lev. In the meantime, unlike other groups of plants, pteridophytes are having distinct and independent sporophyte and gametophyte. In higher plants the dominant sporophytic plant bodies and in lower plant the dominant gametophytic plant bodies are usually taken for screening the bioactivity and for bioactive compounds. Since pteridophytes are peculiar in having independent sporophytic and gametophytic generations, there is a chance for screening bioactivity and bioactive compounds both in sporophytic and gametophytic plant bodies. Bracken gametophytes and sporophytes grown from spores from acyanogenic fronds were found to be cyanogenic. The amount of hydrogen cyanide released differed between gametophytes and sporophytes raised from the same source of spores [4]. Guha *et al.* [5] have studied the antimicrobial activity on both sporophyte and gametophyte of the ferns *Adiantum capillus-veneris*. Studies on antimicrobial activities of crude extracts and extracted phenols from different parts of sporophyte of an epiphytic fern *Arthromeris himalayensis* (Hook.) Ching against *Bacillus subtilis* and *Escherichia coli* showed that both the crude extracts and extracted phenols from sporophytic plant are with antimicrobial activities. Detailed observations revealed that crude extract shows better antimicrobial activity than extracted phenol [6]. The crude extract of the fern *Cyclosorus interruptus* (Willd.) H. Ito is used by indigenous people of New Guinea, to treat burns, cough, malaria and "general sickness" [7]. Farmers in Martandam, Tamil Nadu, India, use this fern as green manure and also to control *Fusarium*-wilt disease in banana. In the present study, the antimicrobial efficacy of different macroscopic (rhizome, croziers, rachis, fertile leaves and sterile leaves) and microscopic (epidermal glands, sporangia and spores) parts of sporophyte and *in vitro* cultured gametophyte of a fern *Cyclosorus interruptus* (Willd.) H. Ito have been screened for antibacterial activity in order to find out the part with maximum antimicrobial efficacy.

EXPERIMENTAL SECTION

The sporophyte of *Cyclosorus interruptus* was collected from Martandam, Kanniyakumari District, Tamil Nadu, India from an open marshy habitat. The collected sporophyte was washed in tap water to remove dust and air dried at room temperature. Different parts of the sporophytes (Figure 1), such as rhizome, roots, rachis, fertile leaves and sterile leaves, croziers were separated and powdered. For sporangia and spores, the fertile fronds were placed in between clean and dry news papers. After few days, the sporangia and spores adhered on the surface of the news paper were collected. Gametophytes were raised through *in vitro* culture of spores in MS medium. All the air dried sporophyte and gametophyte materials were powdered and used for further study. For antibacterial study disc diffusion method was followed. 10 mg of powder from different parts of *Cyclosorus interruptus* was soaked separately in 1 ml of acetone and sterile paper discs soaked in the acetone extract were used to test antibacterial activity against the bacterium *Staphylococcus aureus.* To get the extract of epidermal glands from leaves, the dried leaves were soaked for few minutes in acetone and the extract was concentrated. In acetone, the epidermal glands of leaves completely dissolved without leakage of chemicals from the leaf tissue as studied by Irudayaraj [8]. In order to separate different bioactive compounds of the crude (Acetone) extract, sequential extraction was made with hexane, chloroform and ethyl acetate solvents. For glandular extracts, 50-1000µg concentration range was tested. The minimum inhibitory concentration was carried out according to the method of National Committee for Clinical Laboratory Standards (NCCLS) [9]. The crude acetone extracts were used for MIC studies. The initial test concentration of crude extract was 1 mg/100 µl. It was then serially diluted as follows 15.64, 31.25, 62.5, 125, 250, 500 and 1000 µg/100 µl. Each tube containing 5 ml of bacterial broth was inoculated with 5µL of bacterial suspension containing 108 CFU/ml of bacteria. The plates were incubated for 24 h at 37°C. MIC was determined as the lowest concentration of extract showing no visible growth on the agar plate. All the data were statistically analyzed.



Antibacterial screening of different parts of *Cyclosorus interruptus*

RESULTS AND DISCUSSION

Antibacterial efficacy in different parts of the fern *Cyclosorus interruptus* has been given in table 1 with the size (mm) of inhibition zone (Figure 1). The *in vitro* raised gametophyte shows nil activity. In sporophyte, the macroscopical parts rachis, rhizome, roots and microscopical parts spores and sporangia (without spores) also show nil activity. Antibacterial activity has been observed in mainly leafy parts like croziers (young leaves), sterile and fertile mature leaves. Maximum size of inhibition zone 28 mm has been observed in sterile leaves, followed by 27 in rachis with leaves (27mm) and fertile leaves (23mm). Minimum size of inhibition zone has been observed in croziers (Young leaves) with 18mm. From this study it is concluded that antimicrobial efficacy is seen in leaves only and so the antimicrobial compounds are present mainly in mature sterile leaves. It may be due to the fact that the leaves are main part doing various physiological functions of the plants with the exposure to various biotic and abiotic stresses for which various chemical compounds may be responsible. The rachis, the main axis of the aerial part, gives mainly mechanical support along with conduction of water and minerals to the leafy part –lamina. The synthesized food materials from the leaves are also transported to the underground parts through the rachis. Usually for the development of gametophytes of all the pteridophytes require very narrow optimum physical environmental conditions and the developing or matured gametophytes are not usually exposed to extreme stresses in which they cannot develop at al. So there is no possibility for the presence of diverse secondary metabolites in gametophytes. The *in vitro* cultured gametophytes raised in controlled conditions without any biotic or abiotic stress, there is no chance for induced synthesis of any secondary metabolites. The presence of more antibacterial activity in sterile leaves with 28mm inhibition zone when compared to fertile leaves with 23mm inhibition zone proves that fertile parts such as sporangia and spores do not have much of bioactive compounds as tested individually with nil activity in. There are several reports on the effect of fern spores on growth impairment of human cells [10, 11]. Siman *et al.* [10] have reported that spore extracts of five fern species: *Anemia phyllitidis*, *Dicksonia antarctica*, *Pteridium aquilinum*, *Pteris vittata* and *Sadleria pallida*, induced DNA strand breaks in cultured human premyeloid leukaemia (K562) cells. It is known that human cells are more sensitive than the bacteria, so that the spores of the presently studied fern *Cyclosorus interruptus* have nil activity against the bacterium *Staphylococcus aureus*.

After confirming the maximum antibacterial activity in sterile leaves with 28 mm of inhibition zone, experiment was carried out to know the antibacterial efficacy of the epidermal glands of the leaves by extracting the leaf-glands with acetone (crude) and also by making sequential extraction from the crude extract with hexane, chloroform and ethyl acetate. The results have been given in the table 2. Both the crude (acetone) extract and ethyl acetate extract of the epidermal glands of leaves show comparatively higher antibacterial efficacy against *Staphylococcus aureus* with the inhibition zone from 9 to 15mm in crude extract and from 8 to 13 in ethyl acetate extract. In the case of crude extract maximum inhibition zone, 15mm, is seen in 750 µg and in the case of ethyl acetate extract the maximum inhibition zone 13mm is seen in 100µg itself. The chloroform extract of epidermal glands show antibacterial activity in all the tested concentrations (50-1000µg) with the inhibition zone from 8 to 11mm. The hexane extract of epidermal glands show antibacterial activity only in 100 and 500µg concentrations each with 9mm inhibition zone (Figure 1). The acetone extracts of *Cyclosorus interruptus* revealed the highest antibacterial activity at a minimum concentration 31.255µg/100µl against *S. aureus*.

Parihar *et al.* [12] have observed antibacterial activity in eleven different homosporous ferns with aqueous and alcoholic extracts (10% from fresh leaves) against five different bacteria with the inhibition zone from 0-22mm which include the inhibition zone 9mm for the solvent ethanol alone. The maximum inhibition zone which they observed for the bacterium *Staphylococcus aureus* is 16mm. It is to be noted that all the 11 homosporous ferns which they tested for antibacterial activity are devoid of epidermal glands on leaves. But the present homosporous fern *Cyclosorus interruptus* with epidermal glands on leaves show comparatively higher degree of antibacterial activity.

Hadfield and Dyer [5] have observed that in bracken fern, the amount of hydrogen cyanide released (weight for weight) differed between gametophytes and sporophytes raised from the same source of spores. Those raised from spores taken from different fronds differed significantly, as did those raised from spores from different field sites. Different clones of gametophytes and sporophytes from the same source also differed significantly. For this variation they have interpreted as results of developmental and genetic differences. Ganguly *et al* [6] have observed antibacterial activity in all the tested parts (Rhizome, roots, sterile and fertile leaves) of the sporophytes of an epiphytic fern *Arthromeris himalayensis* (Hook.) Ching. It is reasonable to expect for the presence of various kinds of bioactive compounds in various parts of the high altitude epiphytic fern *Arthromeris himalayensis* which has to

meet various kinds of environmental stresses more severely when compared to the terrestrial fern of the present study *Cyclosorus interruptus* which is growing terrestrially in open marshy habitat in plains or low altitude. Three new coumarin derivatives, compounds 1-3, three new furanocoumarins, compounds 4-6, and a novel dioxocane derivative, compound 7, have been reported from the fern *Cyclosorus interruptus* (Willd.) H. Ito with cytotoxic effects in compounds 1, 5/6 and 7 to a KB cell line [14].

Table 1. Antibacterial efficacy in different parts of the fern *Cyclosorus interruptus*

Parts of the fern *Cyclosorus interruptus*	**Inhibition zone (mm)**
Sporophyte -Rhizome	Nil
Sporophyte- Roots	Nil
Sporophyte- Croziers	**18**
Sporophyte – Rachis with leaves	**27**
Sporophyte –Rachis without leaves	Nil
Sporophyte – Sterile leaves	**28**
Sporophyte – Fertile leaves	**23**
Sporophyte – Sporangia without spores	Nil
Sporophyte - Spores	Nil
In vitro raised gametophyte	Nil

Table 2. Antibacterial activity in different extracts of epidermal glands of *Cyclosorus interruptus*

Extracts of epidermal glands	**Inhibition zone (mm) in different concentrations of Crude extracts (µg)**					
	50	**100**	**250**	**500**	**750**	**1000**
Crude –Acetone Extract	9	10	11	9	15	-
Hexane-Fraction	-	9	-	9	-	-
Chloroform-Fraction	10	9	8	10	11	9
Ethyl Acetate-Fraction	10	13	-	11	12	8

From the present study on screening of antimicrobial efficacy of different parts of the fern, *Cyclosorus interruptus*, it is concluded that the microscopical epidermal glands of the leaves have potential bioactive compounds as observed by Paul Raj *et al.* [3] in the fern *Christella parasitica* (L.) H. Lev. Although each sporangia bear glandular hair on their stalk, the bioactive compounds may not be very low without antibacterial activity. Plants have developed various mechanisms of defense against phytopathogens and phytophagous insects. Two defensive morphological features are trichomes and glands mainly on the leaves. Trichomes may be hair-like or glandular. Glandular trichomes and plant glands synthesize various kinds of secondary metabolites which are toxic to several pathogens and insects. Lipophilic metabolites of glandular trichomes of *Medicago sativa* deter the leafhoppers of Potato [14]. In the glandular trichomes of a medicinal plant *Montanoa tomentosa* two diterpenic acids, kaurenoic and grandiflorenic and twenty-six volatile terpenes have been identified, with the abundance of two terpens, β-eudesmol and valencene [15]. Several bioactive and protective chemicals are synthesized in plant epidermal glands, appendages and other parts [16-19]. The presence of remarkable antibacterial activity in the epidermal glands, when compared to other parts of the medicinal fern *Cyclosorus interruptus* opens the way to exploit the bioactive compounds from epidermal glands of this fern.

REFERENCES

[1] DS Fabricant, NR Farnsworth. *Environmental Health Perspectives*, **2001**; 109 (Suppl. 1), 69-75.
[2] S Jeeva, M Johnson., JS Aparna, V Irudayaraj V. *International Journal of Medicinal and Aromatic Plants* **2011**; 1(2), 107-114.
[3] S Murthy. International *Journal of Nanomedicine* **2007**; 2(2), 129 – 141.
[4] K Paul Raj, V Irudayaraj, M Johnson, D Patric Raja. *Asian Pacific Journal of Tropical Biomedicine*, **2011**; 1(1), 8-11.
[5] P R Hadfield, AF Dyer. *Biochemical Systematics and Ecology* **1988**; 16(1), 9-13.
[6] P Guha, R Mukhopadhyay, P K Pal, K Gupta. *Allelopathy Journal.* **2004**; 13(1), 57-66.
[7] G Ganguly, K Sarkar, S Mukherjee, A Bhattacharjee, R Mukhopadhyay. *Bioresearch Bulletin* **2011**; 5, 311-315.
[8] T Quadri-Spinelli. Biological screening of medicinal plants from Papua New Guinea and phytochemical investigation on the fern *Cyclosorus interruptus.* A dissertation submitted for the degree of Doctor of Natural Sciences, Swiss Federal Institute of Technology, Zurich. **1970**;

[9] V Irudayaraj. *Flora & Fauna* **1996**; 2 (2), 155-157.
[10] NCCLS (National Committee for Clinical Laboratory Standards): Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. In *Approved Standard M100-S12*. Wayne. PA, NCCLS, **2002**;
[11] SE Simán, AC Povey, TH Ward, GP Margison, E Sheffield. *Br. J. Cancer* **2000**; 83(1), 69-73.
[12] SE Siman, E Sheffield. *Fern Gaz.* **2006**; 17(5), 287-291.
[13] P Parihar, L Parihar, A Bohra. *Journal of Microbiology and Antimicrobials* **2010**; 2(2):19-22.
[14] CM Ranger, RK Winter, GE Rottinghaus, EA Backus, MR Ellersieck, DW Johnson. *Journal of Chemical Ecology*, **2004**; 30(10), 1969-1983.
[15] RE Robles-Zepeda, E Lozoya-Gloria, MG López, ML Villarreal, E Ramírez-Chávez, J Molina-Torres. *Fitoterapia* 2009; 80 (1), 12–17.
[16] RD Stipanovic, Protective Chemicals in Plant Epidermal Glands and Appendages. *Plant Resistance to Insects*, Chapter 5, *ACS Symposium Series*, Vol. 208. American Chemical Society 1983; pp 69–100.
[17] M Johnson, EG Wesely, N Selvan, K Chalini. *J. Chem. Pharm. Res.* **2010**; 2(4): 899-906.
[18] M Johnson, A Babu, and V Irudayaraj. *J. Chem. Pharm. Res.* **2011**; 3(1): 715 – 720.
[19] S Suganya, V Irudayaraj, M Johnson. *J. Chem. Pharm. Res*. **2011**; 3(1): 721 – 731.