Anti Inflammatory Activity of Selected Pteridophytes from Western Ghats

Abstract
The present study was aimed to explore the anti-inflammatory potential of selected Pteridophytes from Western Ghats of South India using heat induced haemolytic activity. Stabilization of RBCs membrane was studied to further establish the mechanism of anti-inflammatory action of Cyclosorus interruptus (Wild.) H. Ito., Pityrogramma calomelanos (L.) Link var. calomelanos, Pteris vittata L., Pteris confusa T. G. Walker, Pteris biaurita L., Pteris multiaurita Ag., Adiantum incisum Forssk., Adiantum latifolium Lam., Hemiottis arifolia (Burm.) T. Moore. and Ceratopteris thalictroides (L.) Brong aqueous extracts with varied inhibition. Except A. latifolium and P. confusa all other tested extracts effectively inhibited the heat induced haemolysis. The studied extracts percentage of inhibition were as follows A. incisum > Pityrogramma calomelanos var. calomelanos > Cyclosorus interruptus > P. multiaurita > P. biaurita > H. arifolia = C. thalictroides > P. vittata > P. confusa > A. latifolium. The result of the present study suggested that further studies on the isolation of active principles from the aqueous extracts of Cyclosorus interruptus, Pityrogramma calomelanos var. calomelanos, P. biaurita, P. multiaurita, A. incisum, H. arifolia, and C. thalictroides may bring out an alternative drug for the inflammation.

Keywords: Anti-inflammatory; Pteridophytes; Haemolysis; Antibiotics; Neutrophils

Introduction
Synthetic drugs are employed for the pain and inflammatory administration possesses various side effects, toxic to animals [1] and they are very expensive [2]. In addition, the increase in dominance of multiple drug resistances leads the researcher to develop new anti-inflammatory drugs. Previous studies on the phytotoxicological potentials proved the versatile utility of secondary metabolites [3-7]. Extraction and isolation of various bioactive compounds from plants paved a pathway to discover new active principles with therapeutic application [8-10]. The ancient history reported the utilization of Pteridophytes in Homeopathic, Ayurvedic and Unani medicines as insecticides, antibiotics, food and ornamentation [11]. Lamichhane et al. [12] revealed the anti-inflammatory potential of Cheilanthes albomarginata. Anti-inflammatory activity of Cyathea nilgriensis was reported by Shaya Mary and Mahesh [13]. Ismiarni Komala et al. [14] determined the anti-inflammatory potentials of Nephrolepis falcata and Pyrrrosia lanceolata. With this knowledge the present study was aimed to explore the anti-inflammatory potential of selected Pteridophytes from Western Ghats of South India using heat induced haemolytic activity.

Materials and Methods
The healthy and disease free young sporophytes of selected pteridophytes viz., Cyclosorus interruptus (Wild.) H. Ito., Pityrogramma calomelanos (L.) Link var. calomelanos, Pteris vittata L., Pteris confusa T. G. Walker, Pteris biaurita L., Pteris multiaurita Ag., Adiantum incisum Forssk., Adiantum latifolium Lam., Hemiottis arifolia (Burm.) T. Moore. and Ceratopteris thalictroides (L.) Brong. were collected from their natural habitats, Tamil Nadu, India. The collected sporophytes were washed in the running tap water to remove the debris and unwanted materials. The materials were dried and the excess water was removed using blotting paper. 10gm of young sporophytes were cut into small pieces and boiled with 100 ml of distilled water for 30 min. After 30 min, the aqueous extracts were filtered using Whatman No. 1 filter paper. The filtered extracts were centrifuged at 3000rpm for 10 min. The supernatants were collected and used for anti-inflammatory studies.

Membrane stabilization test
Preparation of red blood cells (RBCs) suspension: Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline [15,16].

Heat induced haemolytic assa: The reaction mixture (2ml) consisted of 1ml of Cyclosorus interruptus, Pityrogramma calomelanos var. calomelanos, P. vittata, P. confusa, P. biaurita, P. multiaurita, A. incisum, A. latifolium, H. arifolia, and C. thalictroides aqueous extracts and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560...
nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the following formula [16,17].

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\% \text{ of inhibition} = \frac{\text{Control ABS} - \text{Sample ABS}}{\text{Control ABS}} \times 100
\]

**Results and Discussion**

Stabilization of RBCs membrane was studied to further establish the mechanism of anti-inflammatory action of *Cyclosorus interruptus*, *Pityrogramma calomelanos* var. *calomelanos*, *P. vittata*, *P. biaurita*, *P. multiaurita*, *A. incisum*, *A. latifolium*, *H. arifolia*, and *C. thalictroides* aqueous extract with varied inhibition (Figure 1). Except *A. latifolium* and *P. confusa* all other tested extracts effectively inhibited the heat induced hemolysis (Figure 1). The results provided evidences for membrane stabilization effect of the selected plant drug as an additional mechanism for their anti-inflammatory effect. Due to the resemblance of RBC membrane with lysosomal membrane, this effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The studied extracts percentage of inhibition were as follows *A. incisum > Pityrogramma calomelanos* var. *calomelanos = Cyclosorus interruptus > P. multiaurita > P. biaurita = H. arifolia = C. thalictroides > P. vittata > P. confusa > A. latifolium*.

![Figure 1: Antiinflammatory Activity of Selected Pteridophytes.](image)

The aqueous extracts of *Cyclosorus interruptus*, *Pityrogramma calomelanos* var. *calomelanos*, *P. vittata*, *P. biaurita*, *P. multiaurita*, *A. incisum*, *H. arifolia*, and *C. thalictroides* inhibited the heat induced haemolysis of RBCs. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. In recent years, the search for plant derived drug with anti-inflammatory properties has been focussed by many researchers due to their potential use in the therapy of various chronic and infectious diseases [18]. The present study also examined anti inflammatory potential of selected plants viz., *Cyclosorus interruptus*, *Pityrogramma calomelanos* var. *calomelanos*, *P. vittata*, *P. biaurita*, *P. multiaurita*, *A. incisum*, *A. latifolium*, *P. confusa*, *H. arifolia*, and *C. thalictroides* using heat induced haemolytic assay. Plant derived phenolic, flavonoids, terpenoids, tannins and saponins possess potent anti inflammatory activity [19-27].

**Conclusion**

The results of the present study showed varied percentage of inhibition, this may be due to the occurrence of phenolic, flavonoids, terpenoids, tannins and saponins in the studied plant extracts. Williams et al. [28] suggested that extracts which can inhibit the denaturation greater than 20% over the range concentration were considered as having anti-inflammatory property [28]. In the present study the aqueous extracts of *Cyclosorus interruptus*, *Pityrogramma calomelanos* var. *calomelanos*, *P. biaurita*, *P. multiaurita*, *A. incisum*, *H. arifolia*, and *C. thalictroides* showed more than 20% of inhibition. The result of the present study suggest that further studies on the isolation of active principles from the aqueous extracts of *Cyclosorus interruptus*, *Pityrogramma calomelanos* var. *calomelanos*, *P. biaurita*, *P. multiaurita*, *A. incisum*, *H. arifolia*, and *C. thalictroides* may bring out an alternative drug for the inflammation.

**Acknowledgement**

None.

**Conflict of Interest**

None.

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