Assessment of Phytochemical, Analgesic and Antioxidant activities of Edible Plant Pteris Vittata L.

***Naznin Shahria[1], AFM Nazmus Sadat[2]**
[1]*Lecturer, Department of Pharmacy, Khwaja Yunus Ali University, Bangladesh*
[2]*Associate Professor, Department of Pharmacy, Khwaja Yunus Ali University, Bangladesh*
**Corresponding author: shahrianaznin@ymail.com*

ABSTRACT

Bangladesh possesses a rich flora of medicinal plants, especially edible plants which are used as vegetables. Edible plants can play a great role as nutraceutical. Bangladeshi nutraceuticals are currently receiving recognition worldwide as being beneficial in various diseases. Thus, the interest of this project was to investigate the nutraceutical stuff Pteris vittata L. providing pharmacological benefits in addition to its basic nutritional values for possible analgesic & anti-oxidant activity. Phytochemical evaluation of the ethanolic extracts of Pteris vittata L. indicates the presence of alkaloids, flavonoids, tannins, gums, carbohydrates and absence of steroids, saponins. The pharmacological interests of these compounds, coupled with the use of this plant in traditional medicine tend to investigate Pteris vittata L. for possible analgesic, anti-oxidant activities. An analgesic activity was investigated on animal model, Swiss Albino mice. Ethanolic extracts of Pteris vittata L. produced 79.55% protection or writhing inhibition in mice at oral doses of 500 mg/kg body weight and 57.95% at 250mg/kg body weight of mice respectively. Ethanolic extract showed anti-oxidant activity in vitro when tested in DPPH scavenging method. The IC_{50} (inhibitory conc. 50%) for ascorbic acid was approximately 12.76µg/ml and for the sample it is more than60.11µg/ml. The above results indicated that the ethanolic extract of the edible plant, Pteris vittata L.has potent analgesic and anti-oxidant effect & may be used as nutraceutical. However,further investigations are necessary to find out the active constituents responsible for this effect.

Keywords: Pteris vittat L., Analgesic activity, Antioxidant activity, Phytochemical test, Writhing reflex.

1. INTRODUCTION

Various pteridophytes have antimicrobial and antioxidant activity, to test the phytochemical and antioxidant activity of these economical plants, the present study was conducted with one of the pteridophyte named as *Pteris vittata L.* **(Bangla name:** Dheki shak,Dhaka shak, **English name:** Chinese ladder fern, brake) commonly occurs all over the country. It is found on almost any calcareous substrate, such as old masonry, sidewalks, building crevices, and nearly every habitat with exposed limestone, notably pinelands. Plant extract is used as demulcent,hypotensive,tonic,antiviral and antibacterial (Sing, 1999). *Pteris vittata* isnative and widespread, found from the east to the south tropicalandSouthern Africa. *Pteris vittata* which grows readily in the wild, is sometimes cultivated, it is grown in gardens for its attractive appearance, or used in pollution control. *Pteris vittata* has unique characteristics of heavy metal accumulation, such as arsenic (Ellis *et al.*,2006).

2. MATERIALS AND METHODS

2.1 Sample collection and extraction

The plant *Pteris vittata* L. was collected from Khulna University, Bangladesh, during the month of October, 2010 on the day time. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no.: 35477) and a voucher specimen was also deposited there. The plant parts were grounded into a fine powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Powder of plant parts was extracted by cold extraction method using ethanol. 250gm of the dried powder was taken in a cleaned jar. After that 95% ethanol (1000ml) was poured into the jar up to 1 inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with aluminium foil was closed properly to resist the entrance of air into the jar. This process was performed for 9 days. The jar was shaken and stirred several times during the process to get better extraction. After the extraction process the extract was filtered by a piece of clean, white cloth. The filtrate was collected in a beaker. The filtrate (ethanolic extracts) obtained was evaporated by Rotary Evaporator and after that it was kept under ceiling fan to evaporate the ethanol completely. It rendered a gummy concentrate (36 gm) of greenish black color. The gummy concentrate was designated as crude extract of ethanol.
Therefore, yield value (%) = 36/250×100% =14.4 %

2.2 Animals
For analgesic activity test, Swiss-Albino mice were collected from animal research branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The mice were of either sex (20-25 gm body weight) and were used for the experiments. The animals were kept in the standard polypropylene cages prior to commencement of the study and provided with standard diets (ICDDR, B formulated). To adjust in the new environment the animals were acclimatized in animal house, Pharmacy Discipline, Khulna University, Khulna under standard Laboratory conditions(relative humidity 55-60%, room temperature 25±20˚ C and 12 hours light: dark cycle) for period of 14 days prior to performing the experiments.

2.3 Drugs
Diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh)

2.4 Phytochemical Tests
The crude extract was subjected to preliminary phytochemical screening for the detection of major groups (Evans, 1989). The extract showed a number of phytochemicals present in it. The plant extract showed the presence of Reducing Sugar, Tannins, Saponins, Gums, flavonoid, Alkaloids, and Glycosides.10% (w/v) Solution of the extract was taken in each test. After that, the extract was used for pharmacological screening.

2.5 Determination of analgesic activity
The analgesic activity of the sample was studied using acetic acid induced writhing model in mice. Experimental animals were selected in random basis and divided into four groups. After that the groups were denoted as Control group, Positive control group and Test group I and Test group II. Each group consisted five mice. Control group was administered 1% Tween-80 and the Positive control group was administered diclofenac sodium at the dose of 25 mg/kg body weight orally. Test group I and Test group II were treated with test sample at the dose of 250 and 500 mg/kg body weight orally. After an interval of thirty minutes to ensure proper absorption of the administered substances the writhing was induced. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. An interval of 5 minutes was given for absorption of acetic acid and after that the number of writhing was counted for 15 minutes. As the animals do not always perform full writhing, the incomplete writhing was taken as half-writhing. That's why two half-writhing were taken as one full writhing. So, total writhing was halved to convert all writhing to full writhing or real writhing (Whittle, 1964; Ahmed *et al.*, 2004).

2.6 *Determination of antioxidantactivity*
2.6.1 Qualitative test
A Suitably diluted stock solutions were spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to

resolve polar and non-polar components of the extract. Antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH free radical (Sadhu *et al.*, 2003). The sample and ascorbic acid were spotted on the TLC plate where ascorbic acid was used as standard. The chromatogram was developed by ascending technique using three types of solvent systems i.e. non polar solvent system (n-Hexane : Acetone=2:1), medium polar solvent system (CHCl3: CH3OH = 5:1) and polar solvent system (CHCl3: CH3OH: H2O = 40:10:1). The solvent system was allowed to move up to a previously marked line. The plates were then air dried. DPPH (1, 1-diphenyl-2-picryl hydrazyl) forms deep pink color when it was dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants.

2.6.2 Quantitative test

The anti-oxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC_{50} (Inhibitory conc. 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta *et al.*, 2003). The formula used for % inhibition ratio is-

% inhibition = (Blank OD-Sample OD/ Blank OD) × 100

Table I: Effect of Ethanolic Extract of *Pteris vittata* L. Acetic Acid Induced Writhing in Mice.

Animal group	**Treatment**	**Writhing Count (%writhing)**	**%Writhing Inhibition**
Control (n=5)	1% tween-80 solution in water	17.6 ± 0.93 (100)	0
Positive Control (n=5)	Diclofenac sodium (25mg/kg)	4.2 ± 0.62* (23.86)	76.14
Test group I (n=5)	Et. Extract (250mg/kg)	7.4 ± 1.44* (42.04)	57.95
Test group I (n=5)	Et. Extract (500mg/kg)	3.6 ± 1.17* (20.45)	79.55

Values are expressed as mean ± SEM, SEM=Standard error of Mean, n=No. of mice, Et.= Ethanolic
*: $P < 0.001$ vs. control.

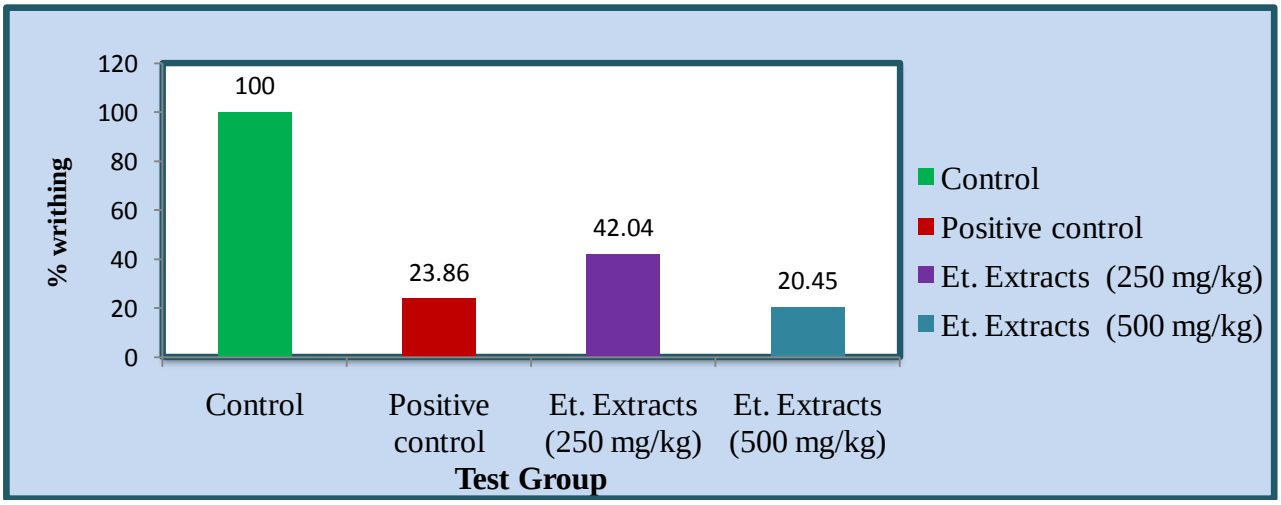

Fig. 1: Effects of ethanolic extracts of *Pteris vittata* L. on acetic acid induced writhing in mice.

Table II: Data on DPPH free radical scavenging activity of Ascorbic Acid(standard) an *Pteris vittata*

Concentration (µg/ml)	Log concentration(µg/ml)	Absorbance (Ascorbic acid)	Absorbance (extract)	%Inhibition (Standard)	%Inhibition (Extract)
0		0.672	0.672	0	0
1	0	0.60	0.62	10.35	7.73
1.57	0.196	0.56	0.6	16.96	10.71
3.13	0.496	0.57	0.58	18.93	13.69
6.25	0.796	0.54	0.57	19.86	15.18
12.5	1.096	0.43	0.54	34.74	19.64
25	1.398	0.13	0.46	79.98	31.55
50	1.699	0.10	0.34	85.11	49.40
100	2	0.07	0.23	89.12	65.77
200	2.301	-	0.21	-	68.75
400	2.602	-	0.189	-	71.88

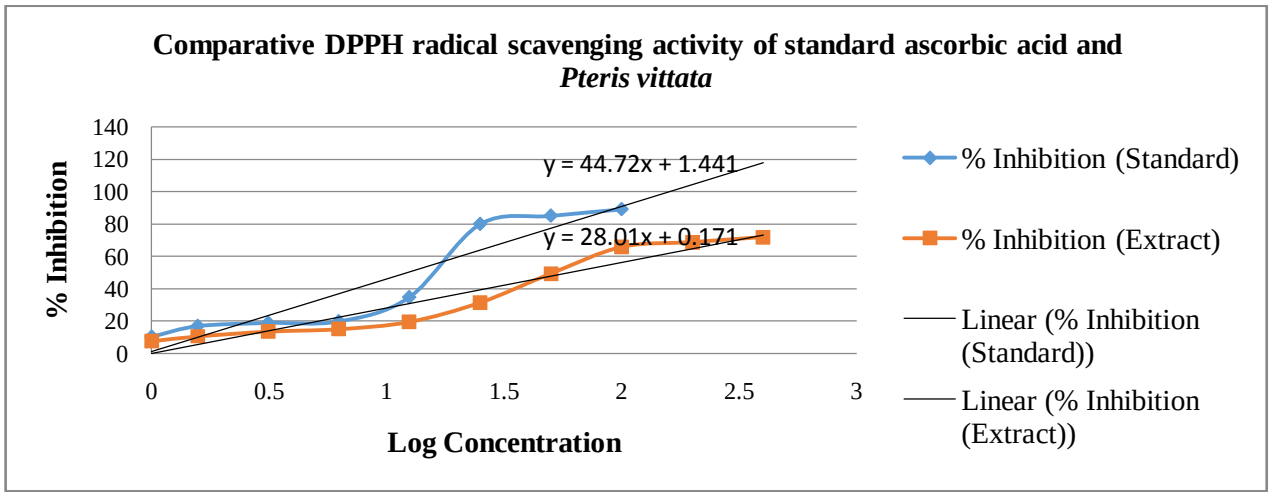

Fig. 2: % Inhibition vs Log Conc. curve of ascorbic acid and ethanolic extract of *Pteris vittata* L.

3. STATISTICAL ANALYSIS

Student's *t*-test was used to determine a significant difference between the control group and experimental groups

4. RESULT

The ethanolic extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups. Analgesic activity of the ethanolic extract was tested by acetic acid induced writhing model in mice. The extract produced 79.55% ($p<0.001$) acetic acid induced writhing inhibition in mice at the dose of 500 mg/kg body weight, which is comparable to diclofenac sodium 76.14% ($p<0.001$) at the dose of 25 mg/kg body weight (Table 1). The extract was also subjected to qualitative and quantitative test for antioxidant activity. The DPPH radical scavenging activity showed IC_{50} at 12.76 and 60.11µg/ml for ascorbic acid and the ethanolic extract of the plant respectively.

5. DISCUSSION

The phytochemical evaluation of ethanolic extract of *Pteris vittata* L. showed the presence of alkaloids, flavonoids, tannins, gums and carbohydrates compounds. Results showed that ethanolic extracts of *Pteris vittata* L. has potent analgesic and antioxidant activity. Traditionally *Pteris vittata*

L. has been used as antibacterial. This work was designed to evaluate the potential analgesic and antioxidant activity of *Pteris vittata L.*

From the previous data it can be assumed that the analgesic activity of the extract was, may be, due to reduction in production of arachidonic acid from phospholipids, or it may inhibit the enzyme system which may be responsible for the production of prostaglandins.

In the TLC-based qualitative antioxidant assay using DPPH , *Pteris vittata* L. showed the free radical scavenging properties indicated by the presence of whitish yellow spot on a purple background on the TLC plate. In the quantitative assay, *Pteris vittata* L. *plant extract* displayed free radical scavenging activity in the DPPH assay which is comparable to that of ascorbic acid a well-known standard antioxidant. The result complies with the previous publications of presence of flavonoids in the plant.

6. CONCLUSION

The ethanolic extracts of *Pteris vittata* L. possess potent analgesic and antioxidant activity. Therefore, *Pteris vittata* L. may be a potential source of neutraceutical with analgesic and antioxidant activity and may contribute to national and /or global health and economy. Further investigations are necessary to find out the active constituents responsible for this effect.

ACKNOWLEDGEMENT

The authors are grateful to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for providing the experimental mice. The authors are also grateful to the authority of Beximco Pharma-ceuticals Ltd. for providing Diclofenac sodium.

REFERENCES

[1] Ahmed F., Das P.K., Islam M.A., Rahman K.M., Rahman M.M and Selim M.S.T. "Antibacterial activity of Cordyline terminalis. Kunth. Leaves". *J. Med. Sci*. 3(5-6), pp. 418-422. 2004

[2] Evans WC. "*Trease and Evans Textbook of Pharmacognosy*", p. 546, 13th ed. Cambridge,1989.

[3] Ellis D.R., Gumaelius L., Indriolo E., Pickerin, I.J., Banks J.A., Salt D.E., "A novel arsenate reductase from the arsenic hyper accumulating fern *Pteris vittata"*. *Plant Physiology*.,vol .141, no.4, pp**.** 1544–1554, Aug. 2006.

[4] Gupta M, Mazumder UK, Kumar RS, Sivahkumar T, Vamis ML.) "In antitumer activity and Antioxidant status of Caesalpinia bonducella against Ehrlich ascites carcinoma in Swiss albino mice". *Journal of Pharmacological Science* vol. 4, no. 2.pp. 177-84. 2003

[5] Sing HB. "potential medicinal pteridophytes of India and their chemical constituents", *Journal of Economic and taxonomic botany*, vol. 23,no.1, pp. 63-78,1999.

[6] Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. "Separation of *Leucas aspera*, a Medicinal Plant of Bangladesh, Guided by Prostaglandin Inhibitory and AntioxidantActivities". *Chemical and Pharmaceutical Buletinl*, vol. 51, no.5 pp. 595-598. 2003.

[7] Whittle BA. "The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics". *British Journal of Pharmacology and Chemotherapy* vol. 22 ,pp. 246. (1964).