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Assessment of antioxidant and analgesic activity of *Acrostichum aureum* Linn. (Family- Pteridaceae)

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Abstract

The plant extract of Acrostichum aureum Linn (Family- Pteridaceae), was themed to phytochemical and pharmacological exploration to determine antioxidant, and analgesic activity to afford an appropriate guide, that may be used in future investigation. Phytochemical exploration of the ethanol plant extract designated the attendance of reducing sugars, alkaloids, glycosides, tannins, flavonoids, gums, and terpenoids. The study of the plant extract illustrated significant free radical scavenging activity in DPPH (1,1-Diphenyl-2-picrylhydrazyl) both qualitatively and quantitatively. Quantitatively, it revealed significant stable DPPH radical scavenging action with the IC_{50} value of 41.95 µg/mL while the IC_{50} value of the standard ascorbic acid was 16.36 µg/mL. In acetic acid induced writhing test in Swiss-Albino mice the ethanol plant extract illustrated statistically significant analgesic activity ($P<0.01$) in dose dependent manner at the doses of 250 and 500 mg/kg-body weight. These outcomes are liable to propose that some chemical constituents are accountable for antioxidant, and analgesic activity of plant extract.

Key words: Acrostichum aureum, Pteridaceae, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), analgesic activity

Introduction

Acrostichum aureum Linn (Family- Pteridaceae), common name: Hudo (Bangladesh), Tiger Fern, Piai raya (Singapore), Golden Leather Fern (South Florida) (1), Swamp Fern, Mangrove Fern, occurs Worldwide in mangrove swamps, salt marshes, canal margins, and low hammocks. It is widely distributed through out S. Florida, Brazil, S. & W. Mexico, Guyanas, Central America, Colombia, Venezuela, Ecuador, Paraguay, Barbados, Trinidad, S. China, Taiwan, Japan, N. Australia, India, Sri Lanka and Bangladesh. (2)

It is an evergreen shrub, can be grown as annual which is locally used as choice of medicinal plant in the treatment of major and minor complaints. Rhizomes are pounded into a paste and used to treat snake-bite, wounds and boils (Malay). Leaves are used to stop bleeding.

In the earlier study, ethanol and acetone extracts of *Acrostichum aureum* Linn. showed anti-implantation activity (3). The spores of *Acrostichum aureum* Linn are potentially allergenic (4). Leaf extracts of *Acrostichum aureum* Linn. (Pteridaceae) was investigated for their total phenolic content (TPC), and antioxidative, tyrosinase inhibiting and antibacterial activities. *A. aureum* showed no antibacterial activity (5). *Acrostichum aureum* produces a large quantity of nicotinic acid glucoside from nicotinamide, but trigonelline formation is very low (6).

In this study, an endeavor was made to defend the conventional uses as per scientific research. Furthermore, the presence of reported compounds was distinguished by using a variety of standard chemical tests. Upon adequate literature review, found that a slight study has been executed to evaluate the rationale uses of this plant in traditional medicine.

In our study, we therefore tried to assess the antioxidant and analgesic activity of the ethanol extract of leaves of *A. aureum*.

Materials and Methods

Plants collection and extraction

Whole plants of *A. aureum* were collected from Sundarbans, Bangladesh, the largest mangrove forest in the world, and branded by the specialists at National Herbarium, Mirpur, Dhaka, Bangladesh (Accession no.: DACB 35553). The plants were shade dried for one week. After adequate drying, the plants were ground into a crude powder with the help of a suitable mechanical grinder and stored in a suitable airtight container and kept in a cool, dark and dry place to avoid any possible fungal attack.

For extracting, cold extraction method was used. About 350 gm of powered material was taken in a suitable container and soaked in 1200 mL of ethanol (99-100%). The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was filtered with clear cotton plug to remove plant garbage. Finally the extract was filtered through filter paper. The ethanol was evaporated with an electric fan at room temperature to find the dried crude extract (yield value 3.73%). Then the dried crude extract was stored in refrigerator.

Test Animals

For the investigation of analgesic activity Swiss-Albino mice of either sex (22-25 gm) were collected from animal resources department of the ICDDR, B (International Center for Diarrhoeal Disease Research, Bangladesh). The mice were kept in the polypropylene cages for acclimatizing by providing with proper rodent foods in animal house, Pharmacy Discipline, Khulna University under the proper laboratory condition (room temperature 25±2°C, relative humidity 55±5%, and 12 hours light: dark cycle) for period of 7 days prior to use.

Chemicals, Reagents and Standard Drugs

Sigma-Aldrich, USA provided 1,1-Diphenyl-2-

pycrylhydrazyl (DPPH). Ascorbic acid and Acetic acid were collected from Merck, Germany. Tween-80 was purchased from Loba Chemie Pvt Ltd, India. Beximco Pharmaceuticals Ltd, Bangladesh supplied Diclofenac Sodium.

Phytochemical Tests

For the identification of foremost functional groups in the crude extract preliminary phytochemical screening was performed (7-9). The plant extract demonstrated the presence of reducing sugars, alkaloids, glycosides, tannins, flavonoids, gums, and terpenoids.

In Vitro Antioxidant Activity Test

Stable free radical DPPH (1,1-Diphenyl-2-pycrylhydrazyl) scavenging method was applied for estimation of antioxidant activity of the ethanol extract of sample both qualitatively and quantitatively (10-13).

Qualitative Test

For qualitative estimation of antioxidant property of test sample Thin Layer Chromatographic (TLC) technique was applied (12). This test was performed with polar, medium polar, and non-polar solvent systems by using TLC plate to determine compounds of different polarities. Bleaching of DPPH (yellow on purple background) radical was observed after spraying 0.02% DPPH in ethanol to TLC plates for the period of 30 min and noted.

Quantitative Test

For quantitative estimation of anti-oxidant potential of the extract was estimated on the basis of their scavenging activity of the stable DPPH radical (12). Firstly 10 mg extract was dissolved to 25 mL with ethanol to prepare 400 µg/mL solution of the sample that was used as stock solution. Then 200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.57 µg/mL solution was prepared by serial dilution technique. Add 2 mL of each sample solution into nine separate test tubes intended for each concentration. Then 6 mL of 0.004% DPPH solution was taken into these test tubes separately, and kept at dark place for 30 minutes for allowing reaction. After that, absorbance was measured by using UV spectrophotometer at 517 nm against blank preparation. Similarly prepared ascorbic acid was used as standard. Calculated the percent inhibition by using following formula:

Percent inhibition = [(Absorbance of blank - Absorbance of sample or standard) / Absorbance of blank] × 100

IC_{50} was found from percent inhibition vs. concentration curve.

Assessment of Analgesic Activity

Acetic acid induced writhing test in mice was used for determining the analgesic activity of the crude sample (12-15). For this test experimental animals (mice) were selected randomly, and split into four represented groups as control, positive control, and test group I and II. Each group contained of six mice. Control group was treated with 1% Tween-80 at the dose of 10 ml/kg body weight in distilled water, and diclofenac sodium at the dose of 25 mg/kg body weight was given to positive control group. Test group I and II were treated at the doses of 250 and 500 mg/kg body weight respectively with the test sample. Oral route was the subjected to these treatments. To ensure proper absorption of the administered substances, thirty minutes interval was given. Then writhing inducer acetic acid solution (0.6%) was injected using intra-peritoneal route to each mice. A five minutes interval was given to proper absorption of administered acetic acid. At that time writhing number was counted for ten minutes. Inhibition of writhing was calculated as percent for both test and control group and compared.

Statistical Analysis

For determining significant differences between

test and control group student's *t*-test was used. Test results were statistically significant when *P*<0.01.

Results

Phytochemical Tests

For identification of different biologically active groups in the ethanol extract, a number of qualitative phytochemical tests were performed and finding results are cited in the Table 1.

Phytochemical groups	Results
Reducing sugars	+
Alkaloids	+
Glycosides	+
Saponins	-
Tannins	+
Steroids	-
Flavonoids	+
Gums	+
Terpenoids	+

+ = Presence - = Absence

Table 1: Phytochemical tests of plant of *Acrostichum aureum*

DPPH Scavenging Activity

The sample demonstrated DPPH scavenging activity with IC_{50} value of 41.95 µg/mL that was greatly comparable to the standard ascorbic acid had 16.36 µg/mL of IC_{50} value.

See Graph 1.

Acetic Acid-Induced Writhing Test

The sample demonstrated 28.86% (*P*<0.001) and 46.77% (*P*<0.01) writhing inhibition at the doses of 250 and 500 mg/kg body weight respectively in a dose dependent manner, that was extremely comparable with standard diclofenac sodium 69.15% (*P*<0.001) at the dose of 25 mg/kg body weight.

see Table 2.

Discussion

The plant extract exhibited some potential phytochemicals, like reducing sugars, alkaloids, glycosides, tannins, flavonoids, gums, and terpenoids.

The antioxidant activity was demonstrated by most popular DPPH scavenging assay method. *This In-vitro* antioxidant activity test shows that *the* investigated plant contains some potential antioxidant compounds. In this method, deep violet color of DPPH is renewed to light yellow color by converting free radical, DPPH to stable DPPH-H by accepting hydrogen radical, or electron. UV spectrophotometer detect an odd electron of DPPH radical at 517 nm against blank and the absorption decreases upon reduction with an antioxidant due to the development of its stable form, DPPH–H (16). It was a concentration dependent manner that was highly analogous to well establish antioxidant ascorbic acid. Flavonoids delineated by the phytochemical investigation of plant extract may be accountable for this antioxidant activity (17,18).

Most widely used acetic acid induced writhing method in mice is applied for assessing *In-vivo* analgesic activity. Peripherally acting analgesic activity of the plant extract is assessed through the activation of locally sensitize peritoneal receptors by inducing writhing. Writhing is induced by the release of endogenous substances. Several endogenous substances like serotonin, bradykinins, histamine, prostaglandins (PGs), and substance P released by acetic acid are liable for producing pain by accelerating nerve endings. The abdominal constrictions response is activated by locally sensitize peritoneal receptors by administering acetic acid is a responsive pathway to approximate the peripherally acting analgesics (19,20). The embarrassment of prostaglandins (PGE_2 and $PGE_{2\alpha}$) synthesis may be the most probable pathway of peripherally acting analgesics (21).

Tannins, reducing sugars, gums, flavonoids, and alkaloids presence in the plant extract may be responsible for the investigated activities, because it is well estabilished that a wide range of bioactivi-

ties are dependable to these phytochemicals (21-26).

Conclusion

Possible antioxidant and analgesic activities was exhibited in the crude plant extract of *Acrostichum aureum* which justify its medicinal activity. To classify fundamental active constituents responsible for bioactivities as well as its mechanism, it should be demand further investigations.

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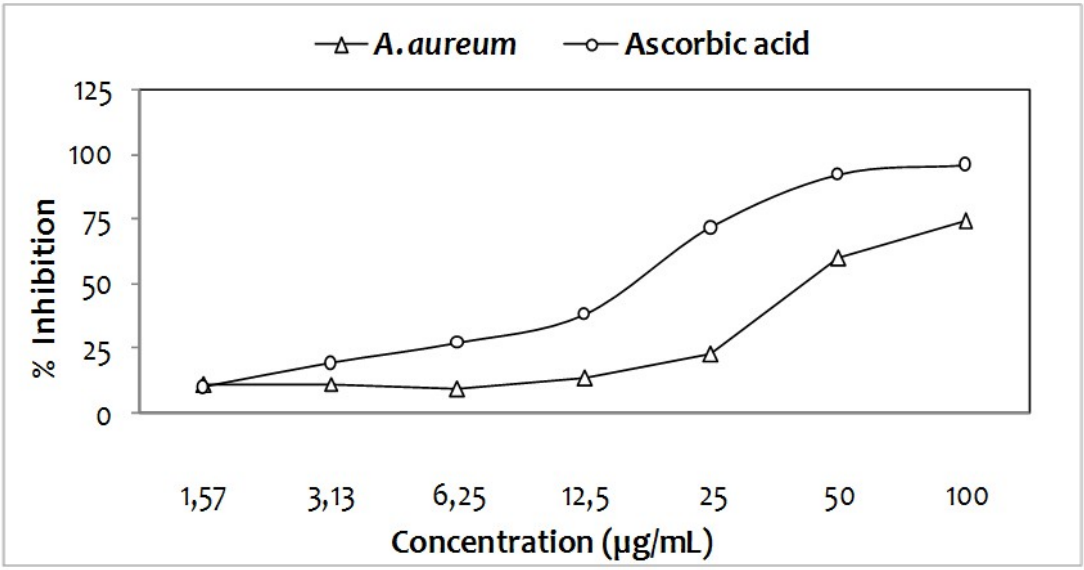

Figure 1: DPPH scavenging activity of *A. aureum*.

Treatment (*n* = 5)	Dose (mg/kg)	No. of writhing	% Inhibition
Control	---	40.20 ± 1.07	---
Diclofenac sodium	25	12.40 ± 1.29**	69.15
Extract	250	28.60 ± 0.93**	28.86
	500	21.40± 1.21*	46.77

Table 2: Effect of *A. aureum* on acetic acid induced writhing in mice
Values are expressed as mean ± SEM, SEM=Standard error of mean, *: $P < 0.01$; **: $P < 0.001$ vs. control, Student's *t*-test.