World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Preliminary phytochemical screening of different solvent extracts of whole plant of *Acrostichum aureum*

Raja S and Ravindranadh K

GITAM Institute of Pharmacy, GITAM University, Visakhapatnam- Andhra Pradesh, India-Pincode-530 045

Received: 29-10-2014 / Revised: 15-11-2014 / Accepted: 21-11-2014

ABSTRACT

Herbal medicines are the major remedy in traditional system of medicine and they are used in medical practices since antiquity. In addition to its ancient historical uses, *Acrostichum aureum* is used in several systems of medicine for a variety of ailments. The main objective of the present study was to investigate the presence of various phytochemicals from the petroleumether, chloroform, ethyl acetate, and methanol extracts of *Acrostichum aureum* whole plant. The four different extracts from whole plant were found to contain triterpinoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins. The generated data from the four different extracts of *Acrostichum aureum* whole plant provided the basis for its wide uses in the traditional & folk medicines.

Key words: *Acrostichum aureum*, Phytochemicals, Folk medicines, Ailments.

INTRODUCTION

Herbal drugs is that the oldest and still the foremost wide used system of medication within the world nowadays. They're created completely from plants. Herbal medicines have many blessings that are fine researched. If we have a tendency to pool the data from various traditions, we've a cure for simply higher than each malady glorious to man. In order to quench the thirst for a new drug for associate degree complaint from herbal origin the plant '*Acrostichum aureum*' has been chosen. *Acrostichum aureum* Linn (Family- Pteridaceae) variously called as Swamp fern, mangrove fern. It's cosmopolitan throughout South American state, Brazil, South & West North American nation, Guyanas, Central America, Colombia, Venezuela, Ecuador, Paraguay, Barbados, Trinidad, and South china, Taiwan, Japan, North Australia, India, Democratic Socialist Republic of Sri Lanka and Bangladesh [1].

It's an evergreen shrub, is grownup as annual that is domestically used as alternative of medicative plant within the treatment of major and minor complaints. The categorization classification of *Acrostichum aureum* was mentioned in below table 1. Ethno medical data suggests that the basis and rootstalk components area unit used as wounds, non healing ulcers and boils [2]. *Acrostichum aureum* is employed to treat pharyngitis, chest pains, hypertrophy, purgative and medicament [3]. A part from this *Acrostichum aureum* has wide selection of pharmacological activities like anti-inflammatory activity [4] antioxidant activity [4] Analgesic activity [5] anti-fertility activity [6] cytotoxicity activity [7]. In the present study, various solvent extracts of whole plant of *Acrostichum aureum* was qualitatively and quantitatively screened for phytochemicals using standard tests.

Table 1: Taxonomical classification of *Acrostichum aureum*

Kingdom	Plantae
Subkingdom	Tracheobionta
Phylum/Division	Pteridophyta
Class	Filicopsida
Order	Polypodiales
Family	Pteridaceae
Genus	*Acrostichum*
Species	*aureum*
Common names	Golden leather Fern, paku laut, mangrove fern, coarse swamp fern, golden leather fern, piairaya, larat, pia

***Corresponding Author Address:** Dr. S. Raja, M. Pharm., Ph.D., Associate Professor, GITAM Institute of Pharmacy, GITAM University, Gandhi Nagar, Rushikonda, Visakhapatnam-530 045, Andhra Pradesh, India, E-mail: sraja61@gmail.com*

MATERIALS AND METHODS

Collection of Plant Material: The plant material was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. Voucher specimen No (GIP-Plant No-004) has retained in GITAM Institute of Pharmacy, GITAM University.

Preparation of plant material: The collected *Acrostichum aureum* whole plant was washed with tap water. The plants were cut in to small pieces and air-dried thoroughly under shade (at room temperature) for 2months to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air - tight container for furthers analysis.

Physicochemical Investigations

Determination of pH range: The pH of different formulations in 1% w/v (1g: 100ml) and 10% w/v (10g: 100ml) of water soluble portions of whole plant powder of *Acrostichum aureum* were determined using standard simple glass electrode pH meter [8].

Loss on drying / Moisture content (Gravimetric determination): Separately place about 1.0g of whole plant powder of the *Acrostichum aureum*, in an accurately weighed moisture disc. For estimation of loss on drying, it was dried at 105°C for 5 hours in an oven, cooled in a desiccators for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air –dried material.

Determination of hot water and ethanol-extractable matter: Individually place about 4.0g of whole plant powder of the *Acrostichum aureum*, in an accurately weighed, glass toppered conical flask. For estimation of hot water -extractable matter, 100ml of distilled water was added to the flask and weighed to obtain the total weight including the flask. The contents were shaken well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and boiled smoothly for 1 hour; cooled and weighed. The flask was readjusted to the original total weight with distilled water and it was shaken well and filtered rapidly through a dry filter. Then 25 ml of the filtrate was transferred to an accurately weighed, tarred flat-bottomed dish (Petri disc) and evaporated to dryness on a water-bath. Finally, it was dried at 105°C for 6 hours in an oven, cooled in desiccators for 30 minutes, and weighed without delay. Same procedure was followed using ethanol instead of distilled water to determine extractable matter in ethanol. The extractable matter was calculated as the content of in mg per gm of air -dried material.

Determination of total ash: Two grams of the whole plant powder of the *Acrostichum aureum*, was placed in a previously ignited (350°C for 1 hour) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550°C for 5hours in a muffle furnace (Nabertherm) until it is white, indicating the absence of carbon. Cooled in desiccators and weighed. Total ash content was calculated in mg per g of air-dried material.

Determination of acid-insoluble ash: 25ml of hydrochloric acid (70g/l) was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter -paper and washed with hot water until the filtrate was neutral. The filter -paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550°C for 3 hours in a muffle furnace to stable weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid-insoluble ash content was calculated as mg per gm of air dried material.

Determination of water-soluble ash: 25 ml of water was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5minutes. Insoluble matter was collected on an ash less filter -paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. Allowed the residue to cool in suitable desiccators for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water - soluble ash content was calculated as mg per g of air-dried material.

Determination of sulfated ash: Ignited a suitable crucible (silica) at 550°C to 650°C for 30 minutes, cooled the crucible in a desiccators (silica gel) and weighed it accurately. One gram of the whole plant powder of the *Acrostichum aureum* was placed in a previously ignited crucible, ignited gently at first, until the substance was thoroughly white. Cooled and moistened the sample with a small amount (usually 1ml) of sulfuric acid (1760g/l) heated gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moistened the residue with a small amount (usually 1ml) of sulfuric acid (1760g/l) TS, heated gently

until white fumes were no longer evolved, and ignited at 800°C +25°C until the residue is completely incinerated. Ensure that flames were not produced at any time during the procedure. Cooled the crucible in a desiccators (silica gel), weighed exactly. This was repeated until the sample reaches a constant weight and calculated the percentage of residue.

Preparation of Extracts: *Acrostichum aureum* plant was refluxed successively with the different solvents like petroleum ether, chloroform, ethylacetate and methanol in a Soxhlet extractor for 72 hrs in batches of 500g each. Every time, the marc was dried before extracting with the next solvent. The excess solvents were removed from all the extracts by vacuum rotary flash evaporator. Further the solvents were concentrated over the hot water bath and finally stored in desiccators for phytochemical analysis.

Preliminary Phytochemical Screening: The preliminary phytochemical screening of the petroleum ether, chloroform, ethylacetate and methanol extracts of whole plant powder of *Acrostichum aureum* were carried out using standard laboratory procedures, to detect the presence of different secondary metabolites (phytochemical constituents) such as alkaloids, flavonoids, saponins, glycosides, tannins, phenols, terpenoids, steroids, protein, quinines, Fixed oils and fats [9-13].

Test for Alkaloid: Mayer's test: 1.2ml of extract was taken in a test tube. 0.2ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff colored precipitate gives positive test for alkaloid.

Wagner's test: 2ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Creation of reddish brown precipitate indicated the positive response for alkaloid.

Test for Tannins: About 2ml of the aqueous extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ Solution were added. Formation of green precipitate was indication of presence of tannins.

Test for Saponins: 5ml of aqueous extract was shaken vigorously with 5ml of distilled water in a test tube and warmed. The development of stable foam was taken as an indication of the presence of saponins.

Test for Flavonoids: To 1 ml of aqueous extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for Terpenoids: Few ml of the organic extract was dissolved in 2ml of chloroform and evaporated to dryness. 2ml of concentrated sulphuric acid was added and heated for about 2 min. Development of a greyish colour indicates the occurrence of terpenoids.

Tests for glycosides

Borntrager's test: Few ml of dil. sulphuric acid added to the test solution. Boiled, filtered and extracted the filtrate with ether or chloroform. Then organic layer was alienated to which ammonia was added, pink red color was produced in organic layer.

Legal test: Extract was dissolved in pyridine; sodium nitroprusside solution was added to it and made alkaline. Pink red color was produced.

Test for carbohydrates: The test solution is combined with a small amount of Molisch's reagent (α-naphthol dissolved in ethanol) in a test tube. After addition, a small amount of conc. sulfuric acid is slowly added down the sides of the sloping test-tube, without mixing, to form a layer. A positive reaction is indicated by appearance of a purple ring at the interface between the acid and test layers indicated that presence of carbohydrates.

Test for Proteins & amino acids

Ninhydrin test: Recently prepared 0.2% Ninhydrin reagent (2 drop) was treated with extract and heated. A blue color developed representing the presence of proteins or peptides or amino acids.

Biuret test: 1 ml of 40% NaOH mixed with 2 drops of 1% copper sulphate to the extract, a violet color indicated the occurrence of proteins.

Tests for steroids

I. A red colour produced in the lower chloroform layer when 2ml of organic extract was dissolved in 2ml of chloroform and 2ml concentrated sulphuric acid was added in it, indicates the presence of steroids.

II. Development of a greenish colour when 2ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

Thin Layer Chromatography: The thin layer chromatography result confirmed the presence of different bioactive compounds. The results and observations were summarized in Table 6. TLC plates were prepared by using Silica Gel-GF 254 as adsorbent. 20gm silica gel-G was mixed with 40ml of distilled water (1:2) to make slurry. The slurry was immediately poured into the plates. Plates were then allowed to air dry for one hour and layer was fixed by drying at 110^0C for one and half hours. Using a micropipette, about 10µml of extracts were loaded gradually over the plate and air dried. The plates were developed in different solvent systems such as ethyl acetate: methanol:

water (100:13.5:10), ethyl acetate: methanol (9:1), benzene: ethyl acetate (95:5), chloroform: methanol (9:1), ethyl acetate: formic acid: acetic acid: water (100:11:11: 26), toluene: dioxane: acetic acid (50:40:10). The different solvent systems showed different R_f value for the same plant extract. The chromatograms were observed under visible light and were photographed.

RESULTS

Organoleptic evaluation: As seen in Table2, both the marketed formulation and household formulation had similar organoleptic properties except for the colour of the both formulation. The Organoleptic characters of the whole plant of *Acrostichum aureum* course powder was tabulated as Table 2.

TABLE 2: Organoleptic properties of whole plant of *Acrostichum aureum*

Parameters	Marketed formulation	In house preparation
Appearance	Powder	Powder
Colour	Yellowish brown	Yellowish green
Odour	Fragnant	Fragnant
Taste	Bitter	Bitter

Physicochemical Investigation: Physicochemical parameters of whole plant powder of *Acrostichum aureum* were estimated based on the methods recommended by World Health Organization (WHO). As apparent from Table 3, the pH of 1% w/ v and 10% w/ v solutions were found to be 05.02 ± 0.02 and 04.77 ± 0.04 respectively. These values were showed not much difference in the pH of water soluble portions of whole plant of *Acrostichum aureum*. Percent weight loss on drying or moisture content value was found to be 9.25 ± 0.33. The less value of moisture content of drugs could prevent content bacterial, fungal or yeast growth through storage [14]. The ash values total ash; water soluble ash, acid insoluble ash and sulfated ash value were found to be 07.16 ± 0.09, 02.45 ± 0.08, 01.69 ± 0.07 and 01.30 ± 0.10 respectively. Ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards [15].The solubility percentage of *Acrostichum aureum* in aqueous hot extraction is higher (27.21 ± 1.27) when compared with ethanolic hot extraction (25.92 ± 0.64). The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent [16].

TABLE 3: Physicochemical parameters of whole plant of *Acrostichum aureum*

PARAMETERS	VALUES
pH of 1% w/v formulation solution	05.02 ± 0.02
pH of 10% w/v formulation solution	04.77 ± 0.04
Loss on drying	9.25 ± 0.33
Total ash value	07.16 ± 0.09
Water soluble ash	02.45 ± 0.08
Acid insoluble ash	01.69 ± 0.07
Sulfated ash value	01.30 ± 0.10
Water soluble (hot) extractive value	27.21 ± 1.27
Ethanol soluble (hot) extractive value	25.92 ± 0.64

Determination of systemic solvent extractive values: The air dried powder of *Acrostichum aureum* plant was extracted by successive extraction with a variety of solvents. The average yield (% w/w) obtained during extraction with petroleum ether, chloroform, ethyl acetate, and methanol was found to be 4.6, 4.2, 3.0 & 5.8 respectively. The average yield during successive extraction of *Acrostichum aureum* plant with four different solvents was tabulated as Table No. 4.

Table 4: Successive extraction of *Acrostichum aureum* plant

Type of extract	Amount of extract (gm)	Yield (% w/w)	Appearance
Petroleum ether	23	4.6	Yellowish black
Chloroform	21	4.2	Brownish black mass
Ethyl acetate	15	3.0	Greenish brown
Methanol	29	5.8	Brownish mass

Preliminary phytochemical screening of *Acrostichum aureum:* It was observed that the preliminary phytochemical screening of *Acrostichum aureum* showed the presence of glycosides, proteins, triterpinoids, and saponins in petroleum ether extract. Chloroform extract revealed the presence of proteins, glycosides, and steroids. Ethylacetate extract showed the presence of proteins, glycosides, steroids and flavonoids, while the methanolic extract showed the presence of proteins, glycosides, steroids, triterpinoids, saponins, and flavonoids. The Preliminary phytochemical screening for various functional groups is tabulated as Table No. 5.

TABLE 5: Phytochemical screening of petroleum ether, chloroform, ethylacetate and methanol extracts of whole plant of *Acrostichum aureum.*

S.No	Tests	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1	Test for carbohydrates				
	Molisch's test	-	-	-	-
2	Test for proteins and amino acids				
	Ninhydrin test	+	+	+	+
	Biuret test	+	+	+	+
3	Test for alkaloids				
	Mayer's test	-	-	-	-
	Wagner's test	-	-	-	-
4	Test for fixed oils and fats				
	Spot test	-	-	_	-
5	Test for glycosides				
	Borntrager's test	+	+	+	+
	Legal test	+	+	+	+
6	Test for Steroids				
	Liebermann burchard test	-	+	+	+
	Salkowski's test	-	+	+	+
7	Test for Triterpinoids				
	Tin+thionyl chloride	+	-	_	+
8	Test for phenolics and tannins				
	Ferric chloride test	-	-	-	-
	Gelatin test	-	-	-	-
	Lead acetate test	-	-	-	-
	Alkaline reagent test	-	-	-	-
9	Test for Saponins				
	Foam test	+	-	-	+
	Haemolysis test	+	-	-	+
10	Test for Flavones and flavonoids				
	Shinoda test	-	-	+	+
	With NaOH	-	-	+	+

(+) Positive (-) Negative

Thin Layer Chromatography: It was observed that Thin Layer Chromatography analysis of *Couroupita guianensis* plant showed the presence of alkaloids & steroids in chloroform extract. On another hand ethyl acetate extract showed the presence of flavonoids. R_f values of solutes separated from the various extracts of *Acrostichum aureum* was tabulated as Table No. 6, 7 & 8.

Table 6. Alkaloids: TLC Studies for Chloroform extract of *Acrostichum aureum*

Solvent system for Chloroform extract *Acrostichum aureum*	**Spraying reagent**	**Colour of spots**	**R_f value**	**Inference**
Ethyl acetate: Methanol: water (100:13.5:10) for Chloroform extract *Acrostichum aureum*	Methanol: Ammonium hydroxide (200:3)	Violet colour	0.06	Presence of alkaloids
Ethyl acetate: Methanol (9:1) for Chloroform extract of *Acrostichum aureum.*	Iodine vapours	Yellow fluorescence	0.64	Presence of alkaloids

It was observed that the thin layer chromatography analysis of *Acrostichum aureum* chloroform extract showed the presence of alkaloids with R_f values of 0.06 & 0.64 in ethyl acetate: methanol: water (100:13.5:10) & ethyl acetate: methanol (9:1) solvent systems respectively. Methanol: ammonium hydroxide (200:3) & Iodine vapours were applied for the detection of alkaloids. Appearance of violet colour and yellow fluorescence indicated the presence of alkaloids in chloroform extract.

Table 7. Steroids: TLC Studies for Chloroform extract of *Acrostichum aureum*

Solvent system for Chloroform extract of *Acrostichum aureum*	**Spraying reagent**	**Colour of spots**	**R_f value**	**Inference**
Benzene: Ethyl acetate (95:5) for Chloroform extract of *Acrostichum aureum*	Iodine vapors	Yellow zone	0.46	Presence of steroids
Chloroform: Methanol (9:1) for Chloroform extract of *Acrostichum aureum.*	UV-light	Intense fluorescence	0.52	Presence of steroids

The thin layer chromatography analysis of *Acrostichum aureum* chloroform extract showed the presence of steroids with R_f values of 0.46 & 0.52 in benzene: ethyl acetate (95:5) chloroform: methanol (9:1) solvent systems correspondingly. Iodine vapours & UV-light were applied for the detection of steroids. Appearance of yellow zone and intense fluorescence indicated the presence of steroids in chloroform extract.

Table 8. Flavanoids: TLC Studies for Ethyl acetate extract of *Acrostichum aureum*

Solvent system for ethylacetate extract of *Acrostichum aureum*	**Spraying reagent**	**Colour of spots**	**R_f value**	**Inference**
Ethyl acetate: formic acid: acetic acid: water (100:11:11: 26) for ethylacetate extract of *Acrostichum aureum*	Liebermann-Burchard reagent	Dark colour	0.24	Presence of flavonoids
Toluene: dioxan: acetic acid (50:40:10) for ethylacetate extract of *Acrostichum aureum.*	Natural products- poly ethylene glycol reagent (NP/PEG)	Intense fluorescence colour	0.44	Presence of flavonoids

The thin layer chromatography analysis of ethyl acetate extract of *Acrostichum aureum* showed the presence of flavonoids with R_f values of 0.24 & 0.44 in Ethyl acetate: formic acid: acetic acid: water (100:11:11: 26) Toluene: dioxane: acetic acid (50:40:10) solvent systems correspondingly. Liebermann-Burchard reagent & Natural products-poly ethylene glycol reagent (NP/PEG) were applied for the detection of flavonoids. Appearance of dark color colour and intense fluorescence colour indicated the presence of flavonoids in ethyl acetate extract.

DISCUSSION

Plants are significant source of potentially bioactive constituents for the improvement of new chemotherapeutic agents. The first step towards this goal, whole plant of *Acrostichum aureum* was subjected to systematic organoleptic evaluation, physicochemical and phytochemical screening to determine the amount of soluble constituents in a given amount of medicinal plant material and are helpful in determining the quality and purity of a crude drug, especially in the powdered form. As seen in Table 2, both the marketed and house hold formulation of whole

plant of *Acrostichum aureum* had similar organoleptic properties except for the taste of the both formulation. Physicochemical parameters of whole plant powder of *Acrostichum aureum* were estimated based on the methods recommended by World Health Organization (WHO). As apparent from Table 3, the pH of 1% w/ v and 10% w/ v solutions were found to be 05.02 ± 0.02 and 04.77 ± 0.04 respectively. These values were showed not much difference in the pH of water soluble portions of whole plant of *Acrostichum aureum*. Percent weight loss on drying or moisture content value was found to be 9.25 ± 0.33. The less value of moisture content of drugs could prevent content bacterial, fungal or yeast growth through storage. The ash values total ash; water soluble ash, acid insoluble ash and sulfated ash value were found to be 07.16 ± 0.09, 02.45 ± 0.08, 01.69 ± 0.07 and 01.30 ± 0.10 respectively. Ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards. The solubility percentage of *Acrostichum aureum* in aqueous hot extraction is higher (27.21 ± 1.27) when compared with ethanolic hot extraction (25.92 ± 0.64). The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent. The average yield during successive extraction of *Acrostichum aureum* plant with four different solvents was tabulated as Table No. 4. As seen in Table 5, it was observed that the preliminary phytochemical screening of *Acrostichum aureum* showed the presence of glycosides, proteins, triterpinoids, and saponins in petroleum ether extract. Chloroform extract revealed the presence of proteins, glycosides, and steroids. Ethylacetate extract showed the presence of proteins, glycosides, steroids and flavonoids, while the methanolic extract showed the presence of proteins, glycosides, steroids, triterpinoids, saponins, and flavonoids. As seen in Table 6, 7, 8 all the extracts were subjected to thin layer chromatography by using different solvent systems. The TLC profiling of all the extracts in ethyl acetate: methanol: water (100:13.5:10), ethyl acetate: methanol (9:1), benzene: ethyl acetate (95:5), chloroform: methanol (9:1), ethyl acetate: formic acid: acetic acid: water (100:11:11: 26), toluene: dioxane: acetic acid (50:40:10) solvent systems confirms the presence of diverse potent bio molecules in these plants. TLC analysis provide an idea about the polarity of various chemical constituents, in a way such that compound showing high R_f value in less polar solvent system have low polarity and with less R_f value have high polarity. These potent bio molecules can be further used for development of different drug in future.

CONCLUSION

Phytochemicals found present in plant extracts of *Acrostichum aureum* indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their some pharmacological activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

ACKNOWLEDGEMENT

The authors are thankful to UGC (New Delhi, India) for providing financial assistance to GITAM institute of pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.

REFERENCES

1. Medina E et al. Soil salinity, sun exposure, and growth of *Acrostichum aureum*, the mangrove fern. Botanical Gazette 1990; 1: 41-49.
2. Burkill IH. A dictionary of economic products of Malay Peninsula. Ministry of agriculture and cooperatives, kualalumpur. 1996; 41.
3. Uddin SJ et al. Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureum*. Pharm Biol. 2012; 50(10):1276-80.
4. Hossain H et al. Anti-inflammatory activity of the ethanolic extract of *Acrostichum aureum* (Linn.) root. Bangladesh Pharm J 2011; 14 (2): 107-9.
5. Khan SA et al. Assessment of antioxidant and analgesic activity of *Acrostichum aureum* Linn. (Family- Pteridaceae). Pharmacol online 2013; 1:166-171.
6. Dhar JD et al. Post-coital anti fertility activity of marine plant *Acrostichum aureum* L in rats. Indian J Med Res.1992; 96: 150-152.
7. Uddin SJ et al. (2S, 3S)-sulfated pterosin C, a cytotoxic sesquiterpene from the Bangladeshi mangrove fern *Acrostichum aureum*. J Nat Prod 2011; 74 (9): 2010-3.
8. Choudhary N et al. An overview of advances in the standardization of herbal drugs. J Pharm Edu Res 2011; 2 (2): 55-70.
9. Saxena PNN et al. Preliminary physico-phytochemical Study of stembark of *Alstonia scholaris* (L) R. BR. - A Medicinal Plant. Int J Pharm Sci Res 2012; 3(4): 1071-1075.
10. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, 1994: 112-120.
11. Farnsworth NR. Biological and phytochemical screening of plants. J Pharma Sci 1996; 55: 225 -276.
12. Tiwari P et al. Phytochemical screening and extraction: A rdedition, Nirali Prakashan, Pune. 1995; 137.
13. Gupta AK et al. Quality standards of Indian medicinal plants, ICMR, New Delhi 2008; 7: 331-339.
14. Pandey MK et al. Standardization of Yakrit Plihantak Churna: An ayurvedic poly herbal formulation. Int J Pharm Sci Res 2012; 3(1):171-176.
15. Swamy P et al. Preliminary pharmacognostical and phytochemical evaluation of *Portulaca quadrifida* Linn. Int J Pharm Tech Res 2010; 2 (3): 1699 -1702.
16. Shwetajain et al. Pharmacognostic and phyto chemical investigations of the leaves of *Zizyphus xylopyrus* (Retz) Wild. Int J Pharm Pharm Sci 2011; 3 (2): 122-125.