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Research Article

PHYSICOCHEMICAL AND PHYTOCHEMICAL CONSTITUTIONS OF RACHIS OF ACROSTICHUM AUREUM L.

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A B S T R A C T

Standardisation of crude drug plays a very important role in identifying the purity and quality of crude drugs. The present analysis discloses standardization which includes moisture content, total ash, acid insoluble ash, water soluble ash, petroleum soluble extractive value, benzene soluble extractive value, ethyl acetate soluble extractive value, ethanol soluble extractive value, methanol soluble extractive value and water soluble extractive value. The highest extractive value was recorded in water soluble extract (6.2%).Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. In the present study, chief phytoconstituents of the *Acrostichum aureum* L (Fern) medicinal plant of Pteridaceae family were identified in order to relate their presence with bioactivities of the plants. This research findings highlights that methanolic extracts of *A. aureum* rachis had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *A. aureum* rachis holds the greatest potential to treat various human diseases and has profound medical applicability.

INTRODUCTION

The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs (Ekor, 2014).

Ferns are generally used in traditional medicine for curing many deadly diseases like skin problems, wounds, cough and reproductive problems as well as to make insect repellent (Nath *et al*., 2016; Bahadori *et al*., 2015). A wide range of medicinal ferns like *Adiantum capillus-veneris*, *Cheilanthes albomarginata, Asplenium nidus* including, *Acrostichum aureum* exist in Asia (Chang *et al*., 2005; Arockia Badhsheeba and Vadivel, 2018). *A. aureum* L is a member of Pteridaceae which is commonly known to the locals as the Swamp Fern or Mangrove Fern.It is an evergreen shrub, found in hostile environment.

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Plant species that thrive in hostile environment replete with bacteria, fungi or virus synthesize defensive natural products against these pathogens, which may also exhibit bactericidal, fungicidal or virucidal activity in human system (Chikezie *et al*., 2015). Several studies have reported the traditional use of *A. aureum's* rhizome for curing wounds, non-healing ulcers, boils, syphilitic ulcers, sore throat, chest pains, elephantiasis, purgative, febrifuge, cloudy urine in women, and rheumatism in Malaysia (Hossan *et al*., 2010), Bangladesh (Pattanaik *et al*., 2008), India (Benjamin and Manickam, 2007) and Yap islands and Micronesia (Defilipps *et al*., 1988).

An understanding of the chemical constituents of plants is a prerequisite for their use in medicine and also for the synthesis of complex chemical substances. Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well. Owing to the significance in the above context, physicochemical and preliminary phytochemical screening of plants are the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world. Thus, the present study deals with the physicochemical and phytochemical screening of *Acrostichumaureum* L. rachis.

MATERIAL AND METHODS

Collection and Identification of the Plant Material

The rachis of *Acrostichum aureum* L were collected from the Puthalam, Kanyakumari District, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu. Voucher specimen (VOCC/VV/Bot/10) was also deposited in the PG & Research Department of Botany, V.O. Chidambaram College, Tuticorin.

Preparation of A.aureum Rachis Powder

Dry conditions are essential to prevent the formation of artifacts as a result of microbial fermentation and subsequent degradation of the plant metabolites. Plant materials are cut or sliced into small pieces to facilitate homogenous drying and prevented from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites resulting to the formation of artifacts. Hence in the present study, the rachis of *A. aureum* were cut into small pieces and were dried in shade and then powder with a mechanical grinder. The powder was passing through sieve number 75 and stored in a labelled airtight container for further studies.

Physicochemical Studies

Physicochemical studies include moisture (loss on drying) contents, ash values and extractive values to determine the quality and purity of the powder of rachis of *A.aureum*.

Moisture (Loss on Drying) Content

About 3g of the air-dried sample was weighed (W_b), into a pre-dried and weighed (W_a) tarred porcelain crucible. The sample was dried in an oven at 100-105°C until two consecutive weighing's (W_c) do not differ by more than 5mg. The moisture content of the sample was calculated with reference to crude air dried drug.

$$\text{Moisture (\%)} = \frac{(W_b - W_C)}{(W_b - W_a)} \times 100$$

Ash Values

Total Ash Value

A silica crucible was heated to redness for 10min and cooled in a desiccator and weighed (W_1). About 3g of the ground air-dried sample was transferred to the crucible and weighed along with the contents accurately (W_2). Sample was ignited gradually in an electrical muffle furnace, increasing the heat to 500-600°C until it is white, indicating the absence of carbon. It was cooled in desiccators and reweighed (W_3). Total ash content was calculated as in equation

$$\text{Total ash (\%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

Acid-insoluble Ash (Silica & Sand content)

10ml of 2*M* HCl was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5min. The watch-glass was washed with 5ml of hot water and the washings were added to the crucible. The insoluble matter was filtered on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, driedon a hotplate and ignited to constant weight (W_4). The residue was allowed to cool in desiccators for 30min, and then weighed. Acid-insoluble ash content was calculated as in equation

$$\text{Acid-insoluble ash (\%)} = \frac{(W_4 - W_1)}{(W_2 - W_1)} \times 100$$

Water Soluble Ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5min. The insoluble matter was collected on an ash less filter-paper. The filter was washed with hot water and then ignited in a crucible for 15min at a temperature not exceeding 450°C. The residue was allowed to cool in desiccators for 30min, and then re-weighed (W_5), calculations were done according to equations

Weight of residue, W_6 (g) = $W_5 - W_1$
Weight of ash W_7 (g) = $W_3 - W_1$
Water-soluble ash (g) = $W_7 - W_6$

$$\text{Water-soluble ash (\%)} = \frac{(W_7 - W_6)}{(W_1)} \times 100$$

Sulphated Ash

A silica crucible was heated to redness for 10min, allowed to cool in desiccators and weighed (W_a). 1g of substance was accurately weighed and transferred to the crucible and weighed along with the contents accurately (W_b). It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1ml concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800 ± 25°C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, and a few drops of concentrated sulfuric acid were added and heated. Ignited as before, allowed to cool, and weighed (W_c). The operation was repeated until two successive weighing does not differ by more than 0.5mg. Calculate the percentage of sulphated ash with reference to the air dried drug.

$$\text{Sulphated ash (\%)} = \frac{(W_c - W_a)}{(W_b - W_a)} \times 100$$

Extractive Values

The extractive values of rachis of *A. aureum* in various solvents like petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and water were determined by employing the method of analysis described in Pharmacopoeia of India (Anonymous, 1996).

About 5g of air-dried rachis powder was taken in a stoppered flask. 100ml of the respective solvent were added, shaken well and allowed to stand for 24h with occasional shaking. Then the content was filtered. 50ml of the filtrate were pipette out into a clean, previously weighed china dish and evaporated on a water bath. Finally it was dried at 105ºCin an oven, cooled in a desiccator and weighed. The percentage of solvent soluble extractive with reference to the air-dried sample was calculated.

Preliminary Phytochemical Screening

Preparation of Plant Extract

The coarse powder was subjected to extraction in 250ml each of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol solvents separately. The coarse powder (10g) of the plant material was weighted and put into the brown glass bottles. Then the solvents were added to it. Then the bottles

were sealed with aluminium foil and kept in laboratory shaker at room temperature, and the bottles were shaken for one week. Finally the extract was filtered through many layers of muslin cloth for coarse filtration. The coarse filtrate was than filtered through What man number 1 filter paper. The obtained filtrate was evaporated in a vacuum rotary evaporator under reduced pressure at 40ºC until the filtrate was reduced to one-third of the starting filtrate volume and the concentrated extracts were further evaporated to get dry extracts. A part of dry extracts were re-dissolved in dimethyl sufloxide (DMSO) and were stored in stopper glass bottles and another part was kept as such in air-tight bottles at 4ºC for further analysis.

Phytochemical Screening

The phytochemical screening gives a general idea regarding the presence of different compounds possessing therapeutic values. The different solvent extracts of *A. aureum* rachis were used for screening the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, catachin, sugar and terpenoids according to standard procedures of Harborne (1973), Brindha *et al*. (1981), Trease and Evans (1989) and Sofowara (1993).

Screening for Alkaloids (Dragendroff's test)

2ml of the extract was mixed with 8ml of 1% HCl, warmed and filtered. Then the filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Screening for Steroids (Liebermann Burchard test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Screening for Coumarin

2ml of the extracts was taken in test tubes. The mouth of the tube was covered with filter paper treated with 3ml of 1*N*NaOH solution. Test tube was placed for few minutes inboiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Screening for Tannins

50mg of various solvent extract powder was dissolved in10ml distilled water and filtered. 1% aqueous iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Screening for Saponin

50mg of the various solvent extract powders was boiled in distilled water in a test tube in boiling water bath and filtered.10ml of the filtrate was mixed with 5ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Screening for Flavonoids (Shinoda Test)

To the extract solution (5ml), added few fragments of magnesium ribbon and concentrated HCl drop wise. Appearance of red or orange red colour indicates the presence of flavonoids.

Screening for Quinone

1ml of the extract was mixed with 1ml of concentrated H_2SO_4. Appearance of red colour shows the presence of Quinone.

Screening for Anthroquinone (Borntrager's test)

50mg of extract powder was taken into a dry test tube and 5ml of chloroform was added and shaken for 5min. The extract was filtered through Whatman No 1 filter paper and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicates the presence of anthroquinone.

Screening for Phenols

The extract powder (50mg) was dissolved in 5 ml of distilled water. To this few drops of 10% ferric chloride solution was added. Appearance of blue or green colour indicates the presence of phenol compounds.

Screening for Protein

The extract powder (50mg) was dissolved in 10ml of distilled water and filtered through Whatman No. 1 filter paper. To the filtrate, 1ml of 40% NaOH was added. Then, 1 or 2 drops of 2% copper sulfate solution was added. Appearance of violet colour indicates the presence of proteins.

Screening for Carbohydrates (Molisch Test)

To 2ml of extracts, 3 drops of α-naphthol (20% in ethanol) was added. Then 1ml of concentrated sulphuric acid was added along the side of the test tube. Reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Screening for Glycosides (Borntrager's test)

Extract powder (50mg) was mixed with concentrated H_2SO_4 (5ml), then it was heated for 3min, afterward it was filtered, after that filtrate was mixed with 0.5ml of 10% NaOH and allowed to stand for 3min. Appearance of reddish brown precipitate indicates the presence of glycosides.

Screening for Reducing Sugar

For the presence of reducing sugars in the extract, Fehling test was performed. An amount of 50mg of the extract powder was taken and added it to the equal volume of boiling Fehling solutions (A and B) in a test tube. A brick- red precipitates indicates the presence of reducing sugar.

Screening for Terpenoids (Salkowski test)

5ml of various solvent extract was mixed in 2ml of chloroform followed by the careful addition of 3ml concentrated sulfuric acid (H_2SO_4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

RESULTS AND DISCUSSION

The dry powder is evaluated for its physiochemical parameters like moisture, total ash, water soluble ash, acid soluble ash, sulphated ash and different solvent extractive values (Table 1). The physicochemical parameters are mainly used in judging the purity and quality of the drug. Moisture is one of the major factors responsible for the deterioration of drugs and herbal

formulations. The moisture promotes the degradation processes caused by enzymes, development of microorganisms, oxidation and hydrolysis reactions. This study recorded moisture content of 9.27% which is deemed to be good as water content in herbal drugs should not be greater than 14% (www.intechopen.com).

Table 1 Physicochemical constants of *A. aureum* rachis

Constants	Percentage
Moisture contents	9.27 ± 0.30
Total ash contents	0.75 ± 0.04
Water soluble ash	2.43 ± 0.05
Acid soluble ash	1.67 ± 0.04
Sulphated ash	1.24 + 0.06
Extractive values	
Petroleum ether	2.3±0.02
Benzene	1.4±0.07
Ethyl acetate	2.6±0.01
Methanol	4.2±0.04
Ethanol	2.7±0.05
Water	6.2±0.01

A high ash value is indicative of contamination, substitution or adulteration by minerals. The residue remaining after incineration of plant material is the total ash or ash value. Ash value represents both physiological ash and non-physiological ash. Physiological ash is derived from plant tissue due to biochemical processes while non-physiological ash consist of residue of the extraneous matter (such as sand, soil etc.) deliberately or non-deliberately adhering to plant sample itself. Physiological ash gets dissolved in the dilute acid; while, some of the non-physiological ash remains undissolved. Total ash may compose of carbonates, phosphates, nitrates, sulphates, chlorides, and silicates of various metals which are taken up from the soil or environment (Abdu *et al*., 2015). In the present investigation, the total ash content of *A. aureum* rachis air-dried power is found to be 7.50%, which is less than the maximum acceptable limit of total ash (14%) recommended by European Pharmacopoeia (Vaikosen and Alade, 2011).

Acid insoluble ash is a part of total ash and measures the amount of silica present especially as sand and siliceous earth in the samples. The values also indicate the magnitude of presence of oxalates, carbonates, phosphates, oxides and silicates. Therefore, the values are indices of excellence of herbal remedies. Water-soluble ash is the part of the total ash content, which is soluble in water. This study shows 2.43% water soluble ash in *A. aureum* rachis.

Preliminary phytochemical screening of plants is important in the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs. In the present study, the presence of sixteen phytochemicals were screened in the petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol extracts of the rachis of *A. aureum* and the results are shown in Table 2.

Table 2 Preliminary phytochemical screening of *A. aureum* rachis

Phytochemical	Name of the extract				
	Petroleum ether	Benzene	Ethyl acetate	Ethanol	Methanol
Alkaloids	-	-	-	-	-
Steroids	-	+	+	+	+
Coumarins	-	-	-	-	-
Tannins	-	-	-	-	-
Saponins	+	-	-	+	+
Flavonoids	-	-	+	-	+
Quinone	-	-	-	-	-
Anthroquinones	-	-	-	-	-
Phenols	+	+	+	+	+
Proteins	+	+	+	+	+
Carbohydrates	-	-	-	-	-
Glycosides	+	+	+	+	+
Reducing Sugars	-	-	-	-	-
Terpenoids	+	-	-	+	+

+ indicates the presence of the phytochemical; - indicates the absence of the phytochemical

Presence or absence of certain important bioactive compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. In India traditional communities like tribal and rural populations are frequently using the crude extracts of local plants for medicinal and other purposes. Crude extracts and medicines manufactured on the principles of natural compounds even by pharmaceutical companies, may lead to large scale exposure of humans to natural products. The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in popular medicine. Hence, in the present study, the crude extracts obtained by petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol solvents were screened for the presence of phytochemicals.

The petroleum ether extract showed the presence of saponins, phenols, proteins, glycosides and terpenoids. The benzene extract showed the presence of steroids, phenols, proteins and glycosides. The ethyl acetate extract showed the presence of steroids, flavonoids, phenols, proteins and glycosides. The ethanol extract showed the presence of steroids, saponins, phenols, proteins, glycosides and terpenoids. The methanol extract showed the presence of steroids, saponins, flavonoids, phenols, proteins, glycosidesand terpenoids. Among the phytochemicals, phenols and glycosides were detected in all the presently investigated solvent extracts.

This research findings highlights that methanolic extracts of *A. aureum* rachis had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *A. aureum* rachis holds the great potential to treat various human diseases and has profound medical applicability. Smitha and Vadivel (2019) also reported that the methanolic extracts of *Ceratopteris thalictroides*, a pteridophyte, also had the highest number of phytochemicals. The presence of steroids, saponins, flavonoids, phenols, proteins, glycosides and terpenoids in methanolic extracts of *A. aureum* rachis signals their therapeutic potential. Hossaini *et al* (2011) reported that ethanolic extract of *A. aureum* root contains glycosides, saponins, flavonoids, steroids, fatty acids and long-chain hydrocarbon compounds.

Saponins are naturally occurring surface-active glycosides with a distinctive foaming characteristic (Desaia *et al*., 2009). Saponins are bitter in taste and in recent years, they have received considerable attention because of their various biological activities including hepatoprotective, anti-ulcer, anti-tumor, antimicrobial, adjuvant and anti-inflammatory activities. Saponins have health benefits such as cholesterol lowering and anticancer properties (Gurfinkel and Rao, 2003). Recent research has established saponins as the active components in many herbal medicines (Liu and Henkel, 2002) and highlighted their contributions to the health benefits of foods such as soybeans (Kerwin, 2004) and garlic (Matsuura,

2001). The presence of these compounds, therefore, suggests good pharmacological potential for *A. aureum*.

Flavonoids are secondary metabolite known to rich in pharmacological properties such as anti-oxidative, anti-fungal, anti-inflammatory and diuretic actions. Flavonoids are considered favoured bio-compounds as chemotaxonomic markers in plants because they show large structural diversity and are chemically stable (Kumar and Pandey, 2013). The flavonoids extracted from ferns have shown promising potential in view of their anti-cancer, anti-microbial (Nithya *et al*., 2016), anti-oxidant (Maruzzella, 2005) and anti-inflammatory activities (Singh *et al*., 2008) of the potential use in treating diabetes (Xiao, 2015). Flavonoids derived from *Cheilanthes tenuifolia* (fern) possess potent anti-cancerous, anti-bacterial, anti-oxidant activities that are responsible for their chemo-preventive potential against bacteria (Jarial *et al*., 2018). Phenolics have biological and pharmacological properties such as anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities (Wojdylo *et al*., 2007).

CONCLUSION

Since the plant *A. aureum* has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidantactivity and this activity will help in preventing a numberof diseases through free radical scavenging activity (Arockia Badhsheeba and Vadivel, 2018).The present analyses suggest that *A. aureum* (fern) contains potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. Thus, it's also adding new compounds to the ever increasing canvas of secondary metabolites acting as fountains of health.

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