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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical screening and Amino acid profiling of a mangrove fern *Acrostichum aureum* L

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ABSTRACT
Medicinal plants are a source of many potent and powerful drugs. Acrostichum aureum L belonging to the family Pteridaceae is well known for curing a wide variety of diseases. This work aims to evaluate the preliminary phytochemical screening in the methanolic extract and amino acid profiling of Acrostichum aureum L. Whole parts of plant were collected from natural population of Thiruvananthapuram and processed. Methanolic extracts were obtained using Soxhlet apparatus. Phytochemical screening showed the presence of alkaloids, flavonoids, terpenoids and saponins. Amino acids are the building blocks of proteins and found in significant quantities. This study concludes that Acrostichum aureum is rich in Phytochemicals and amino acids. Further research is required to know more about the bioactive compounds of Acrostichum aureum which can be used as therapeutics tools.
Keywords: *Acrostichum aureum L, Pteridaceae, Alkaloids, Flavanoids and Terpenoids.*

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INTRODUCTION

Acrostichum aureum L is a large terrestrial plant of the family Pteridaceae which are associated with mangroves prevalent in flooded areas during rainy season at high tides. This plant is usually seen in Kerala [1]. This plant is used as a worm remedy and as an astringent in haemorrhage because of its anthelmintic and styptic property [2]. The natural chemicals present in plants accredit the positive or negative health effects are called phytochemicals [3]. The medicinal properties of plants are determined by their phytochemical constituents [4]. Proteins are very essential nutrients for human beings. Amino acids serve as building blocks for proteins and play a crucial role in various biological processes, such as gene expression, homeostasis, and hormone synthesis. Additionally, they also act as precursors for the production of secondary metabolites [5]. The aim of present study is phytochemical screening of whole parts in methanolic extract of *Acrostichum aureum* and amino acid analysis.

MATERIAL AND METHODS

Collection of Plant material and processing

Fresh plant material was gathered from the natural population of Thiruvananthapuram district in Kerala. The collected plant material underwent multiple washes with clean water to eliminate any dust particles. Subsequently, the material was dried in the shade, finely ground into a powder, and safely stored in an airtight container within a refrigerator for subsequent experiments.

Preparation of extracts.

50 gm of leaf, rachis, rhizome and root powder was successively extracted in 500 ml of solvents (Hexane, Ethyl acetate, Chloroform, Methanol and distilled water) using Soxhlet apparatus. The obtained extraction was filtered and subjected to vacuum evaporator. Then the final crude methanolic extract was used for further phytochemical analysis.

Preliminary phytochemical screening.

Methanolic extract of leaf, rachis, rhizome and root was phytochemically screened for the qualitative analysis of major classes of secondary metabolites by slight modifications based on standard procedures [6].

Test for Alkaloids.
The analysis involved conducting Dragendorff and Wagner's tests. The extract was heated with a 2% H2SO4 solution for a duration of 2 minutes. Subsequently, the mixture was filtered, and a few drops of Dragendorff's reagent were introduced. The formation of an orange-brown precipitate indicated the presence of alkaloids. Furthermore, the extract underwent warming with 2% H2SO4 for 2 minutes, followed by filtration. Next, a few drops of Wagner's reagent were added, and the appearance of a reddish-brown precipitate confirmed the existence of alkaloids.

Test for Flavanoids.
A small amount of the extract was heated with 10 ml of ethyl acetate for 3 minutes. Subsequently, the mixture underwent separate filtration, and the resulting filtrates were utilized for the subsequent tests.
Ammonium Test: The filtrate was vigorously mixed with 1 ml of diluted ammonia solution (1%). The layers were observed to remain separate. The presence of flavonoids was indicated by a yellow coloration in the ammonia layer.
Alkaline NaOH Test: Two milliliters of the extract were combined with a few drops of 20% NaOH solution. The appearance of a vibrant yellow color, which disappeared upon the addition of diluted HCl, suggested the presence of flavonoids.
Shinoda Test: One milliliter of the extract was obtained and mixed with a few magnesium turnings, followed by the gradual addition of concentrated HCl. The presence of flavonoids was confirmed by the development of a pinkish coloration.

Test for Terpenoids.
Salkowski Test: 2 ml of chloroform were added to the extract, followed by the careful addition of 3 ml of concentrated H2SO4 to create a distinct layer. A positive indication for terpenoids was observed by the formation of a reddish-brown coloration at the interface.

Test for Saponins
Frothing Test: To assess the presence of saponins, various extracts were diluted with 4 ml of distilled water. The resulting mixture was vigorously shaken until a stable froth formed. The presence of froth indicated the presence of saponins.

Amino acid profiling.
This method employed to estimate the quantity of amino acids [7]. A specific amount of fresh tissue was finely chopped and immersed in boiled 80% methanol for 10 minutes, followed by a reflux process. The resulting mixture was homogenized and then subjected to filtration and centrifugation at 7500 rpm for 10 minutes. The supernatant, containing the desired components, was collected and adjusted to a known volume using 80% methanol. A measured portion of this solution was pipetted, to which 5 ml of Ninhydrin-reagent was added. After thorough mixing, the mixture was boiled in a water bath for 10 minutes. Subsequently, it was allowed to cool under running water, and the absorbance was measured at 570 nm against a blank sample. The resulting absorbance value was then converted to milligrams per gram (mg g-1) using established constants for different amino acids derived from the total amino acid content.

RESULTS AND DISCUSSION.

Preliminary Phytochemical analysis.
The preliminary phytochemical screening of *Acrostichum aureum* indicates that the plant is quite rich in phytochemicals (Table 1) such as alkaloids, flavonoids, terpenoids and saponins in crude methanolic extract. The crude methanolic extract of leaf, rachis and rhizome revealed the presence of alkaloids, which are one of the most extensive categories of compounds synthesized by plants. These alkaloids are derived from nitrogenous substances, either with or without the prior degradation of proteins. Plants contain alkaloids that have been utilized for treating a wide range of conditions including pain, cough, diarrhea, cancer, poor blood circulation, malaria, and other ailments The methanolic extracts of A. aureum were found to contain flavonoids, as observed in the study. Flavonoids have been shown to possess antimicrobial properties, making them beneficial for wound healing and the treatment of skin diseases [8]. Additionally, flavonoids have been recognized for their hypoglycemic, antioxidant, anti-inflammatory, and anticarcinogenic activities [9]. In A. aureum, terpenoids are found in various bio parts and are typically soluble in lipids. They are primarily located in the cytoplasm and encompass essential oils, carotenoids, gibberellins, abscisins, sterols, saponins, and latex. Terpenoids serve several functions, including the regulation of growth, facilitation of photosynthesis, and provision of distinctive colors. Certain terpenoids with 10 or 15 carbon atoms are known as essential oils due to their volatility, which contributes to the characteristic odor of specific species [10]. *A. aureum* were found to contain saponins, which are natural compounds known for their surfactant properties and unique foaming characteristics. Saponins are considered natural detergents or surfactants and have significant industrial applications, such as in mining and ore separation, as well as in the preparation of emulsions for photographic films. Moreover, they are

widely utilized in the cosmetics industry due to their emollient effects and their beneficial antifungal and antibacterial properties, which contribute to their importance in cosmetic applications [11].

Amino acid profiling.

Amino acid profile of *A. aureum* was presented in Table 2. The amino acid content was more in the leaf followed by stem and root.

Tyrosine

The tyrosine content in *A. aureum* showed an increase in leaf (25.678 mg g^{-1}) followed by rachis (23.902 mg g^{-1}), rhizome (23.138 mg g^{-1}) and root (16.602 mg g^{-1}). Tyrosine serves as a building block for neurotransmitters and has the ability to elevate plasma neurotransmitter levels [12]. Additionally, Tyrosine can be beneficial in alleviating stress, combating cold conditions, and reducing fatigue [13].

Phenyl alanine

The phenyl alanine content in leaf (1.763 mg g^{-1}) of *A. aureum* was found to be greater than rachis (1.641 mg g^{-1}), rhizome (1.684 mg g^{-1}) and root (1.143 mg g^{-1}). It acts as a precursor for the production of tyrosine, which is then utilized in the synthesis of several important monoamine signaling molecules such as dopamine, norepinephrine, and epinephrine. Additionally, phenylalanine is involved in the formation of melanin, a pigment responsible for skin coloration

Serine

The order of distribution of serine content in *A. aureum* was leaf >rhizome>rachis>root. The serine content was high in leaf (4.107 mg g^{-1}) followed by rhizome (4.053 mg g^{-1}), rachis (3.823 mg g^{-1}) and root (0.15034 mg g^{-1}). Serine plays a vital role in metabolism by actively engaging in the synthesis of purines and pyrimidines. It serves as a precursor for various amino acids, including glycine, cysteine, and tryptophan in bacteria, while also contributing to the formation of essential metabolites such as sphingolipids and folate.

Glycine

The glycine content in *A. aureum* varied in leaf, rachis, rhizome and root. Leaf possessed high glycine content (1.841 mg g^{-1}) than rhizome (1.754 mg g^{-1}), rachis (1.713 mg g^{-1}) and root (1.158 mg g^{-1}). Glycine primarily serves as a precursor to proteins, playing a crucial role in their formation. Research suggests that consuming 3000 milligrams of glycine before bedtime can enhance sleep quality [14]. Furthermore, glycine has shown promise as an effective treatment for schizophrenia [15].

Aspartic acid

The Aspartic acid content of the leaf of *A. aureum* was high compared to stem and root. The aspartic acid content was high in leaf (11.926 mg g^{-1}) followed by rhizome (11.769 mg g^{-1}), rachis (11.283 mg g^{-1}) and root (7.983 mg g^{-1}). Aspartate plays a vital role in the urea cycle and contributes to gluconeogenesis. It serves as a carrier of reducing equivalents in the malate-aspartate shuttle, which involves the conversion of aspartate to oxaloacetate.

Proline

The proline content of A. *aureum* showed a decrease from leaves to root (17.481 mg g^{-1}, 16.66, 15.975 and 0.639 mg g^{-1}). Proline stands out among the 20 amino acids that form proteins due to its exceptional characteristics. Unlike the others, proline features a secondary amine as its amine nitrogen is attached to two alkyl groups, granting it a unique distinction. The distinctive cyclic structure present in its side chain grants proline remarkable conformational rigidity, setting it apart from other amino acids.

Cysteine

The cysteine content of *A. aureum* varied from leaves (15.637 mg g^{-1}), rachis (14.581 mg g^{-1}), rhizome (14.928 mg g^{-1}) and root (10.128 mg g^{-1}). Cysteine biosynthesis is of vital importance in plants as it serves as a pivotal process for assimilating inorganic sulfur from the surroundings. This process exclusively produces the metabolic sulfide donor necessary for generating various essential compounds such as methionine, glutathione, phytochelatins, iron-sulfur clusters, vitamin cofactors, and numerous secondary metabolites.

Iso leucine

The isoleucine content in *A. aureum* varied from leaves to root. A gradual decrease was noticed from leaf (2.127 mg g^{-1}), rhizome (2.027 mg g^{-1}) rachis (1.980 mg g^{-1}), and in root (1.375 mg g^{-1}). Isoleucine is obtained from pyruvic acid and is classified as an essential amino acid for humans. This designation arises from the fact that the human body cannot synthesize it from other compounds, necessitating its intake through dietary sources. Isoleucine belongs to the aliphatic group of amino acids, based on its distinctive R group.

CONCLUSION

In this research, the phytochemical analysis and amino acid profiling of *Acrostichum aureum* L. were investigated. The study revealed the presence of significant bioactive compounds like alkaloids, flavonoids, terpenoids, and saponins. The leaf of the plant exhibited a higher concentration of amino acids. Notably, the amino acids Tyrosine, Phenylalanine, Serine, Glycine, Aspartic acid, Proline, Cysteine, and Isoleucine were detected in substantial amounts. Further exploration of additional bioactive components will enhance our understanding of the therapeutic applications associated with *Acrostichum aureum* L.

Table 1: Preliminary phytochemical screening in methanolic extract of *Acrostichum aureum* L.

Methanolic extract	Alkaloids	Flavonoids	Terpenoids	Saponins
Leaf	+	+	+	+
Rachis	+	-	+	-
Root	-	+	+	+
Rhizome	+	+	+	+

Table 2: Amino acid profiling in *Acrostichum aureum* L.

Amino acid	Leaf (mg g^{-1})	Rachis (mg g^{-1})	Rhizome (mg g^{-1})	Root (mg g^{-1})
Glycine	1.841	1.713	1.754	1.128
Serine	4.107	3.827	4.053	2.655
Cysteine	15.637	14.581	14.928	10.128
Aspartic acid	11.926	11.283	11.767	7983
Proline	17.481	15.975	16.666	11.302
Isoleucine	2.127	1.980	2.027	1.375
Tyrosine	25.678	23.902	23.138	16.602
Phenyl alanine	1.763	1.641	1.684	1.143

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research.

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