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REGULAR ARTICLE

PHARMACOGNOSTICAL STUDIES ON THREE *ASPLENIUM* SPECIES

V. Irudayaraj and M. Johnson*

Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu– 627 002, India

SUMMARY

The present study was aimed to study the pharmacongonstical characters viz., morphological and physico-chemical characteristics of three rare medicinally important spleenworts viz. *Asplenium affine* Swartz, *Asplenium decrescens* Kunze and *Asplenium zenkeranum* Kunze. The present study revealed the presence of alkaloids, triterpenes, and flavonoids in *Asplenium* species. Based on the Thin Layer chromatogram, interspecific relationship was assessed. *A. affine* and *A. decrescens* showed 42% of similarity coefficient and *A. zenkeranum* was varied from *A. affine* and *A. decrescens* with 36% variance.

Key words: Pharmacognosy; *Asplenium*; Phytochemicals;

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*Corresponding Author, Email: ptcjohnson@gmail.com, Tel: + 91 97869 24334; Fax: + -91 462 2561 765

1. Introduction

One of the oldest human activities has been the study of plants as sources of food, shelter, and clothing and as medicines for curing various diseases. The science of medicinal plants in India was at the peak of its glory during the Vedic period (2000 BC - 800 BC). ''Vrikesayurveda'' a treatise written by Parasara during the pre - Christian era was the most authentic text book for students of indigenous medicine in India. The name of Charaka, Sustra and Dhanvanthri, Ayurvedic physicians are well known and they never need any formal introduction. Late with the establishment of Mughal's rule in India from the 13th centaury onward, the Medieval Greeco-Arab system, more commonly known as the Unani - Tibia system which also advocated the use of plants as medicine also become popular. However, these systems become less important, because of the lack of proper identification of the medicinal plants [1]. There is a need for documentation of research work carried out on traditional medicines [2]. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostical studies [3]. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological , anatomical and biochemical characteristics [4].

Asplenium is a genus of about 700 species of ferns, often treated as the only genus in the family Aspleniaceae, though other authors consider *Hymenasplenium* separate. Both the scientific name and the common name "spleenwort" are derived from an old belief, based on the doctrine of signatures, that the fern was useful for ailments of the spleen, due to the spleen-shaped sori on the backs of the fronds. "-wort" is an ancient English term that simply means "plant" (compare German *-wurz*). There are several reports to show the medicinal uses and properties of *Asplenium* species. *Asplenium trichomanes* L. is used as an expectorant, anti-cough remedy, laxative, emmenagogue, abortifacient and for irregular menses. [5]. However, available

literature [5-12] revealed that there is no pharmacognostical study on the Indian species of *Asplenium* species; hence the present investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like extractive values; fluorescence analysis and preliminary phytochemical analysis of three medicinally important spleenwort species viz. *Asplenium affine* Swartz, *Asplenium decrescens* Kunze and *Asplenium zenkeranum* Kunze.

2. Materials and Methods

Plant materials *Asplenium affine* Swartz, *Asplenium decrescens* Kunze and *Asplenium zenkeranum* Kunze (Aspleniaceae) were collected from Kakachi stream, Upper Kothayar Hills, Tamil Nadu region of Western Ghats, South India. The plants were identified and confirmed by Pteridophyte Flora of the Western Ghats, South India [2]. The powder of the shade-dried material was used for Physico-chemical analysis by following the methods of Pharmacopoeia India [13]. Various ash values and extractive values were determined by following standard method [14, 15]. Preliminary phytochemical screening was done by the following the method of Brindha *et al.* [16]. Thin layer chromatography of leaves extracts were also carried out using the standard method. For the inter-specific relationship studies, the TLC chromatogram was converted into a "1" and "0" matrix, to indicate the presence or absence of the Rf Values, respectively. Genetic similarities (GS) were estimated according to Nei and Li [17]. To demonstrate the interspecific relationship, a dendrogram was constructed by UPGMA using NTSYSpc-2.0 software.

3. Results and Discussion

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance [18, 19]. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. In the present study also we provided a detailed morphological description and illustration for three *Asplenium* species viz., *A. affine* (Fig.1 A), *A. decrescens* (Fig.1 B) and *A. zenkeranum* (Fig.1 C).

Asplenium affine Swartz

Habit

Herb, very rare species, confined to Tirunelveli hills, no record elsewhere from India.

Morphology

Rhizome, short creeping, upto 1.25 cm thick, densely scaly at the apex, scales ovate – laneceolate, about 3 × 0.5 mm, dark brown with narrow pale brown, one-two-layer membraneous border, apex acuminate margin with small outgrowths; the membranaceous border usually withered off in mature scales, stipes crowded, upto 23 × 0.4 cm, dark brown, rounded below, grooved above, glabrous, fronds, bipinnate, apical and / or lateral primary pinnae often bear vegetative buds; apex, acuminate, pinnae about 10 pairs, spreading, shortly stalked, subopposite (or) alternate, spaced upto 4cm; largest pinnae12 × 4 cm, lanceolate, apex acuminate, acroscopic base tunnate, basiscopic base cuneate; pinule upto 7 pairs, alternate, sessile, anadromous, ovate (or) oblong, about 2.5 × 0.8 cm, apex rounded (or) subacute, acroscopic base subtruncate (or) cuneate, often sub-auriculate, basiscopic base narrowly cuneate, decurrent, margin crenate, veins slightly distinct below, slightly raised above, dichotomously branched. Sori are

median (or) submedian along the veinlets, about 5 × 1 mm, indusiate, indusia pale brown, entire, spores are ellipsoid (or) planoconvex, 50 × 35µm, dark brown with broad, pale brown, undulate anastomosed winged perispore (Fig.1.A).

Asplenium decrescens *Kunze*

Habitat

Herbs, they are common in Tirunelveli hills and on Kerala Ghats. This species is confined to Sri Lanka and South India (13). This species was found in a variety of habitats. Thus they are seen as terrestrial along road cuttings in dry place (or) as lithophytes along stream and stream banks (or) epiphyte inside the forest between 1050 – 2100 m, in partially (or) fully shaded (or) exposed places.

Morphology

Rhizome long creeping, 0.5 cm thick, densely covered by scales, stipes scattered, dark brown, rounded below, grooved above, glabrous and polished. Fronds are simple pinnate. Pinnae 15 – 25 pairs, shortly stalked, opposite (or) subopposite in the lower part of lamina, alternate above, 6 – 10 × 1 – 2.5 cm, lanceolate, falcate, dimidiate with long, cardate, acuminate apex, margin irregularly lobed one-third to two-third way to the centre; lobes oblong, apices serrate, veins distinct below, obscure above, forked, free, reaching the margin, pinnae pale green, upper surface almost glabrous, lower surface with long, soft, light brown scales. Scales are also borne at the junction of pinnae and rachis, texture subcoriaceous. Sori are along the costa, spores reniform, 38 × 19 µm, with densely reticulate ridges (Fig.1.B).

Asplenium zenkeranum *Kunze*

Habitat

Herb, lithophyte on fully shaded forest floor by stream banks between 1100 – 1200 m. Occasional on Tirunelveli hills, rare on Kerala Ghats. This species is confined to Sri Lanka and South India.

Morphology

Rhizome, short creeping (or) prostrate, about 4 cm thick, densely scaly at the apex, scales ovate to ovate-lanceolate, about 8 × 1.5 mm, dark brown with pale brown border, acute (or) acuminate, gland tipped, margin fimbriate. Stipes crowded, dark brown below, green above when fresh. Fronds are simply pinnate, imparipinnate, pinnae about 15 pairs, spreading, shortly stalked, basal 3 to 5 pairs opposite, next few pairs subopposite, rest of the pairs alternate, about 4 cm apart, pinnae 20 × 3.5 cm, oblong lanceolate or lanceolate, abruptly narrow towards distal most part, apex acuminate, pinnae dark green, glabrous above and below. Apical and lateral pinnae usually bear vegetative buds with leaves and roots. Sori linear along basal part of the macroscopic veinlet forming two oblique rows along the costa, about 10 × 2 mm, indusia yellowish-green when young, entire, glabrous. Spore ellipsed (or) spherical, about 50 µm in diameter, dark brown with broad undulate, anastomosed, winged perispore (Fig.1.C).

Physical constants

Results of ash analysis of the dried leaves of three *Asplenium* species have been presented in table-1.

Table - 1: Ash analysis of three *Asplenium* species

Contents	*A. affine*	*A decrescens*	*A. zenkeranum*
Total ash	6.92%	5.17%	6.28%
Acid insoluble ash	0.87%	1.05%	1.42%
Moisture content	72.12%	86.34%	87.40%

Fluorescence analysis

Powdered dried leaves of three *Asplenium* species were treated with different chemicals and solvents over night in room temperature and the colour of the extracts was observed under ordinary light and UV-Light (Table- 2 and 3).

Table - 2: Fluorescent analysis of extracts from inorganic solvents

Chemicals	*A. affine*		*A decrescens*		*A. zenkeranum*	
	Ordinary	UV	Ordinary	UV	Ordinary	UV
1N HCl	Light brow	Pale brown	Light brown	Pale green	Light brown	Pale green
aq.1N NaOH	Brown	Intense green	Brown	Purple green	Brown	Intense green
1N NaOH in methanol	Intense green	Parrot green	Light green	Parrot green	Intense green	Parrot green
50% HNO_3	Pale golden yellow	Pale green	Green yellow	Pale green	Golden yellow	Parrot green
50% H_2SO_4	Dark brown	Purplish brown	Dark brown	Dark purple brown	Dark brown	Dark purple brown

Extractive values

The powdered leaves of *A. affine, A. decrescens* and *A. zenkeranum* were extracted with non polar and polar solvents separately. The results of the extractive values have been presented in table - 4. The maximum extractive value was observed in acetone extract of *A. zenkeranum* (14.0%), while minimum extractive value was observed in carbon tetrachloride extract of the same species.

For fluorescence analysis, the powders of *Asplenium* species were treated various chemical reagents to give different colours. This may be helpful to identify the purity of the drug. The results of various types of ash may provide a basis to identify the quality and purity of the drug. The present study on micro morphological features, other physical values and chemical parameters on three species of *Asplenium* will helpful to identify the correct species of the plant when there is possibility of adulteration.

Table - 3: Fluorescent analysis of extracts from organic solvents

Solvents	*A. affine*		*A decrescens*		*A. zenkeranum*	
	Ordinary	UV	Ordinary	UV	Ordinary	UV
Distilled water	Reddish brown	Purple green	Reddish brown	Purple green	Reddish brown	Purple green
Acetone	Intense green	Purple green	Intense green	Purple green	Intense green	Purple green
Ethyl acetate	Intense green	Purple green	Intense green	Purple green	Intense green	Purple green
Carbon tetrachloride	Purple brown	Purple brown	Purple brown	Purple brown	Purple brown	Purple brown
Benzene	Intense green	Purple green	Intense green	Purple green	Intense green	Purple green
Pet. ether	Purple green	Purple green	Purple green	Purple green	Purple green	Purple green
Ethanol	Intense green	Pale green	Intense green	Pale green	Intense green	Pale green
Chloroform	Purple green	Purple green	Purple green	Purple green	Purple green	Purple green

Table - 4: Extractive values of three *Asplenium* species

Solvents	*A. affine* (%)	*A decrescens* (%)	*A. zenkeranum* (%)
Petroleum ether	4.6	4.6	5.47
Benzene	5.34	3.74	8.0
Ethylacetate	2.0	2.0	5.0
Chloroform	6.32	5.40	5.01
Carbon tetrachloride	6.0	6.0	2.0
Acetone	11.5*	11.5*	14.0*
Ethanol	6.8	6.8	3.9
Aqueous	3.4	2.94	3.51

*Extractive values are significant

Phytochemistry

The preliminary examination of the different extract of the whole plant of three species of *Asplenium* by various tests suggested the presence of major components viz., alkaloids, flavonoids, triterpenoids and glycoside (Table 5). Terpenoids exert wide spectrum of activities such as anti-malarial, anti-plasmodial, anti-tuberculosis, anti-fungal and anti-cancer. Alkaloids are significant for the protection and survival of plants because they ensure their survival against microorganisms (anti-bacterial and anti-fungal activities) insects and herbivores and also against other plants by means of allelopathically active chemicals. They function as signal compounds, attract pollinating or seed dispersing animals and represents adaptive characters that have been subject to natural selection during the evolution [26, 27]. *In vitro* studies of flavonoids have displayed anti-allergic, anti-inflammatory, anti-microbial, anti-cancer activities and antioxidant activity [22-.25] Consumers and food manufacturers have become interested in flavonoids for their possible medicinal properties, especially their putative role in prevention of cancers and cardiovascular diseases. Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. Cardiac glycosides are used therapeutically mainly in the treatment of cardiac failure due to their anti-arrhythmic effects. Now a day, Standardization of herbal drugs is a topic of great concern. The presences of cardiac glycosides were shown by all the extracts excluding benzene and water extract. Ethanolic and acetone extracts gave positive results for alkaloids, carbohydrates, flavonoids and cardiac glycosides. In addition to that, saponins and phenolic compounds are present in ethanol extract and sterols are present in acetone extracts. Chloroform extract gave positive result for alkaloids, carbohydrates and cardiac glycosides. Benzene extract was shown the positive result for carbohydrates. Petroleum ether extract showed the presence of carbohydrates and cardiac glycosides. Water extracts showed the presence of carbohydrates and phenolic compounds.

There are several reports to show the presence of various kinds of flavonoids in various species of *Asplenium.* A new flavonol glycoside from the fronds of *Asplenium antiquum* has been isolated and the structure has been elucidated as mearnsetin 3,7-di-*O*-α-L-rhamnopyranoside by means of spectral analysis [10]. Chemotaxonomical survey on different species of *Asplenium* i.e *A. foreziense*, *A. fontanum* subsp. *fontanum* and subsp. *pseudofontanum*, *A. obovatum* subsp. *obovatum* var. *obovatum* and var. *protobillotii*, *A. obovatum* subsp. *lanceolatum*, and *A. incisum* shows the presence of the flavonoid kaempferol 3-*O*-gentiobioside as the major constituent in all the species [11]. Phytochemical investigations on the aerial parts of *Asplenium scolopendrium* led to the isolation of four terpenoids, the structures of which were assigned as lutein, (6S,9S)-roseoside, icariside B_2, and picrionoside A using spectroscopic data [12].

Table - 5: Phytochemical screening of three *Asplenium* species

Solvents	*A. affine*				*A. decrescens*				*A. zenkeranum*			
	Alkaloids	Triterpene	Flavonoids	Glycoside	Alkaloids	Triterpene	Flavonoids	Glycoside	Alkaloids	Triterpene	Flavonoids	Glycoside
Water	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	-	+	-	-	-	+	-	-	+	+	-	-
Ethyl acetate	-	-	-	-	-	-	-	-	+	-	-	+
Carbon tetrachloride	-	-	+	-	+	-	-	-	-	-	-	-
Benzene	-	-	-	-	-	-	-	-	+	-	-	-
Petroleum ether	-	+	+	-	-	+	-	-	-	+	-	-
Ethanol	+	-	+	+	+	-	+	+	+	-	+	+
Chloroform	+	+	-	-	+	+	-	-	+	+	-	-

TLC analysis of acetone extracts of thee *Asplenium* species

The chosen extracts of *Asplenium* species (*A. affine, A decrescens and A. zenkeranum)* Were subjected to TLC analysis on moving running solvents system of Toluene: Ethylacetate (93:7) and 7 spots Rf values such as 0.25 (Yellow), 0.33 (Bluish green), 0.39 (Yellow), 0.43 (Grey), 0.51 (Grey), 0.60 (Yellow), 0.63 (Grey) were observed in *A. affine.* In *A decrescens* 7 spots were observed and the Rf values are 0.16 (Pale green), 0.25 (Green), 0.29 (Green), 0.39 (Yellow), 0.43 (Grey), 0.54 (Yellow), 0.60 (Grey). *A. zenkeranum* showed 6 spots with Rf values 0.29 (Green), 0.32 (Bluish green), 0.39 (Yellow), 0.54 (Grey), 0.63 (Grey) and 0.69 (Grey)(Table–6).

Table - 6: Inter-specific relationship analysis of *Asplenium* species based on TLC phytochemicals (Rf values)

Spots	*A. affine*	*A decrescens*	*A. zenkeranum*
1	0	1	0
2	1	1	0
3	0	1	1
4	0	0	1
5	1	0	0
6	1	1	1
7	1	1	0
8	1	0	0
9	0	1	1
10	1	1	0
11	1	0	1
12	0	0	1
Total	7	7	6

Interspecific relationship analysis of *Asplenium* species

For the inter-specific relationship studies, the TLC chromatogram was converted into a "1" and "0" matrix, to indicate the presence or absence of the Rf Values, respectively (Table - 6). A dendrogram was constructed NTSYSpc-2.0 software, the dendrogram revealed the inter-relationships between the selected three spleenworts. The dendrogram shown that the single point origination, it was confirmed that all the three species belongs to the family Aspleniaceae. The dendrogram shown that two clusters, of which Cluster 1 showed 42% similarity coefficient, cluster 1 showed two branches C_1 N^1B_1 was *A. affine* and $C_1N^1B_2$ was *A. decrescens*. Cluster 2 (C_2N^1) *A. zenkeranum*,

showed 36% similarity coefficient Fig. 5. It has been found that in some species of *Asplenium*, the chloroplast genome has evolved in complex and highly unusual ways. This makes standard cladistic analyses unsuited to resolve the phylogeny of that particular group of ferns, and even very sophisticated computational phylogenetics methods yield little information.

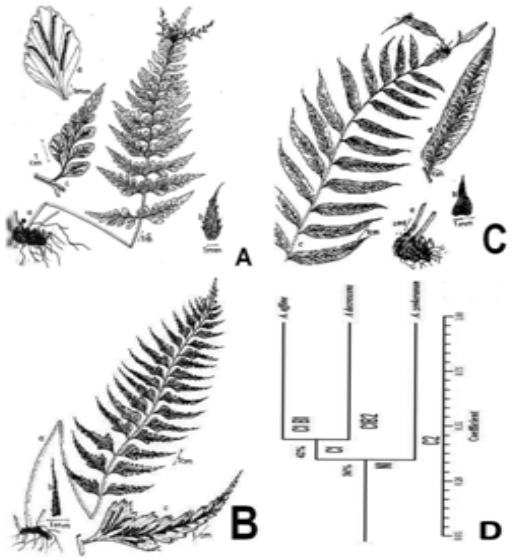

Fig.1.

- *A. Asplenium affine* Swartz, a. Habit, b. Rhizome scale, c. Primary pinna, d. Pinnule enlarged showing venation and sori.
- *B.* Asplenium decrescens *Kunze, a. Habit, b. Rhizome scale, c. Pinna showing venation pattern and sori*
- C. Asplenium zenkeranum *Kunze a. Rhizome (Short creeping), b. Rhizome scale, c. Lamina, d. Pinna showing venation pattern and sori*
- D. Dendrogram of *Asplenium* based on the chromatogram of TLC using NTSYSpc-2.0 software

The present study shows the major difference in some pharmacongonstical characters between the selected three species *A. affine, A. decrescens* and *A. zenkeranum*. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [26]. In the present investigation we observed the high extractive values in acetone compared to other solvents (Table – 4). Preliminary phytochemical analysis indicated presence of alkaloids, triterpenoids, glycoside and flavonoids. In last four decades the scientists are keen to evaluate many plant drugs used in medicinal folk lore. It is due to their specific healing properties, healthy action and non-toxic effects [27].

The present study on pharmacognostical characteristics and preliminary phytochemical screening of *Asplenium* species provide valuable information which may help in authenticating the genuine *Asplenium* along with the nature of phytoconstituents present in it. The above studies provide information regarding their identification and chemical constituents which may be useful for the standardization and preparation of monograph of *Asplenium*. The constituents of *Asplenium* may have several medicinal properties and can be utilized for the treatment of various diseases. Further research on this particular *Asplenium* species may help in the isolation of therapeutically potent compounds which can be finally be subjected to pharmacological activities and clinical trials, thus leading to opening up new avenues in the use of natural products for therapeutic purpose. The pharmacognostical characters reported in this work can serve as a valuable source of information and provide suitable diagnostic tool for the standardization as well as identification of adulterants in future investigation or application. It will also be immense using carrying out further research and revalidation of its use. The microscopic features could help in laying down micro morphological standards as per WHO guidelines for authentication of the original drug.

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