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Inter Specific Variation Studies on Cyathea Species Using Phyto-chemical and Fluorescence Analysis

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Research Article

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ABSTRACT

Tree ferns belonging to the family Cyatheaceae are well known for their beautiful huge foliage throughout the world. It is the second largest living fern group among the pteridophytes. It comprises about 500 species classified into four genera viz., *Cyathea, Alsophila, Cnemidaria* and *Sphaeropteris*. Among these, 200 species are neotropical, the majority belonging to the genus *Cyathea*. Tree ferns always attract researchers and botanists because of their notable morphology, wide geographical distribution and local endemism. In the present study, phytochemical profile on various extracts of selected *Cyathea* species were analyzed using qualitative chemical tests. Preliminary phytochemical analysis was performed in five extracts of *C. nilgirensis, C. gigantea* and *C. crinita* to detect the metabolites presence or absence. All the tested three plant species showed significant indication of phenolics, flavonoids, tannins, cardiac glycosides, terpenoids, steroids, saponins and alkaloids. The results paved a way to find the chemical constituents of the studied *Cyathea* species which may lead to quantitative estimation and also in locating the source of bioactive principles for various pharmacological properties.

INTRODUCTION

Myriads of living organisms described in terms of species and varying individuals of a species are distributed on the planet earth. Mankind is almost totally dependent on plants for their basic requirements. About 1.9 million plant species have been described so far, the estimated total number of species on earth exceeds 11 million [1]. Pteridophytes, the pioneer colonizers on earth, are one of the ubiquitous vegetation about 350 million years ago and they dominated the land in the Carboniferous period. It possesses simple organization and is unique in being characterized by cryptogamic mode of reproduction. They are very conspicuous and gorgeous elements of biodiversity which occurs in various kinds of habitats ranging from sea level to mountain top and tropical to subpolar regions [2].

In natural systems, plants face excess of antagonist and possess myriads of multiple defense mechanisms by which they are able to cope with various kinds of biotic and abiotic stress [3]. Plants produce a high diversity of natural products or secondary metabolites which are important sources of various fine chemicals with a prominent function in protecting the plants from predators and microbial pathogens on the basis of their toxic nature, repellence to herbivores and microbes [4]. Phytochemicals are bioactive substances of plants that are used directly or as intermediates for the production of pharmaceuticals and have been associated in the protection of human health against various chronic degenerative diseases. Phytomedicines have always been a major component of traditional systems of healing in developing countries, which have also been an integral part of their history and culture [5].

Pteridophytes form a neglected group of plants in biodiversity as far as their economic value is concerned. This is not because

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of the misunderstood fact that they lack any economic utility, but the real fact is enough attention has not been paid towards assessing the potentialities of ferns and fern allies towards human welfare. However, with the introduction of ethnobotany by Hershberger [6] for the study of relationship which exists between peoples of primitive societies and their plant environment, many attempts were made on the study of relationships of pteridophytes with man, particularly for their medicinal value. Theophrastus (327-287 BC) and Dioscorides (50 AD) mentioned the medicinal attributes of certain ferns. They have been successfully used in the homoeopathic, ayurvedic, unani and tribal systems of medicines [7, 8]. Pith of *Cyathea nilgirensis* Holttum is used against snake bite [9]. It has central analgesic activity [10] and anti-diabetic activity [11]. Fresh rhizome of *Cyathea gigantea* (Wall. ex Hook.) Holttum mixed with powdered black pepper seeds are taken orally with milk twice a day for one week in empty stomach against white discharges [8]. Rhizome is used against snake bite. Aerial parts of *C. gigantea* have anti-inflammatory properties [12]. Fronds of *C. gigantea* are used for decoration by tribes. The stem is cut and used for the cultivation of epiphytic orchids [13]. Rhizome and sporophyll of *Cyathea crinita* (Hook.) Copel. have antibacterial properties [9, 14]. *Gopalakrishnan et al* screened the presence of starch, total sugars, aminoacids, proteins, chlorophyll a, chlorophyll b, total chlorophylls and carotenoids on the lamina of *C. crinita, C. gigantea* and *C. nilgirensis* [15]. They also studied the distribution of various aminoacids present in the chloroform and ethanolic extracts using the mobile phase n-butanol: acetic acid: water (12:3:5). Janakiraman and Johnson [16, 17] studied the UV-Vis and FT-IR spectroscopic profile of *C. nilgirensis*, *C. gigantea* and *C. crinita*. With deep concern and relevance to Indian medicinal pteridophytes and sense of realization about its medicinal value, the present research work was undertaken to reveal the qualitative phytochemical profile and physico-chemical characters of *C. nilgirensis, C. gigantea* and *C. crinita*.

MATERIALS AND METHODS

Collection of plant materials

Specimens for the present study were collected from different parts of Tamil Nadu, South India. *Cyathea nilgirensis* Holttum were harvested in and around Kakkachi stream, Tirunelveli hills, *Cyathea gigantea* (Wall. ex. Hook.) Holttum from the road sides near Nadugani, Nilgiris hills and *Cyathea crinita* (Hook.) Copel. from the Anglade Institute of Natural History, Shenbaganur, Palni hills, Western Ghats, South India. The plants were identified based on the "Pteridophyte Flora of the Western Ghats, South India" [18]. Herbarium specimens were deposited in the St. Xavier's College Herbarium (XCH), Palayamkottai (*C. nilgirensis* - XCH 25423; *C. gigantea* - XCH 25422 and *C. crinita* - XCH 25424).

Preparation of extracts

The collected species of *Cyathea* were thoroughly washed with tap water followed by distilled water. They were blotted on the blotting paper and shade dried at room temperature under dark. The shade dried plant samples were ground to fine powder using mechanical grinder. 30 g powdered samples were extracted successively with 180 ml of petroleum ether, chloroform, acetone and ethanol using soxhlet extractor for 8-12 hours at a temperature not exceeding the boiling point. The aqueous extracts were prepared directly by boiling the powder with distilled water for 3 hours and filtered using Whatman No.1 filter paper. The extracts were concentrated in a vacuum at 40 °C using rotary evaporator.

Qualitative phytochemical screening

The different qualitative chemical tests were performed on various extracts of selected *Cyathea* species according to the method described by Harborne to detect the presence of phytoconstituents viz., steroids, alkaloids, phenolic compounds, cardiac glycosides, flavonoids, saponins, tannins, anthraquinone, coumarins, catechin, terpenoids and aminoacids [19].

Physico-chemical parameters

Extractive values and fluorescence analysis were determined by following the standard method [20]. The concentrates were transferred to pre-weighed glass vials and completely dried under a stream of air. Aqueous extracts were collected into pre-weighed glass jars and freeze-dried. The percentage yield of each dried extract in terms of the starting plant material was determined. It was then stored in the dark at 7 °C until required for analysis. Fluorescent characteristics of the plant powders as such and after treating them with various chemical reagents viz., Con. H_2SO_4, Con. HCl, CH_3COOH, NaOH and 5% $FeCl_3$ were observed in visible light as well as under UV radiation at 365 nm. The changes in colour were recorded.

RESULTS

Preliminary phytochemical analysis of twelve different metabolites was performed in five extracts of *C. nilgirensis, C. gigantea* and *C. crinita.* All the three plant species showed significant indication about the presence of various bioactive secondary metabolites viz., steroids, alkaloids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and terpenoids (Table 1). Coumarin was present only in *C. gigantea* and catechin demonstrated its existence only in *C. nilgirensis.* Anthraquinone and amino acids failed to show their presence in all the tested extracts of selected *Cyathea* species.

C. nilgirensis showed positive result for phenolics and cardiac glycosides in all the tested five extracts followed by flavonoids and terpenoids in four extracts. Steroids, alkaloids and saponins were present in three different extracts of *C. nilgirensis* whereas tannins and catechin showed its occurrence only in ethanolic extracts of *C. nilgirensis*. Among the five different extracts of *C.*

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nilgirensis, ethanolic extracts of *C. nilgirensis* illustrated the maximum frequency (75%) of metabolites presence followed by acetone 50%, petroleum ether 41.66%, chloroform 33.33% and 25% in aqueous extracts.

Table 1. Phytochemical constituents of studied *Cyathea* species.

Metabolites	*C. nilgirensis*					*C. gigantea*					*C. crinita*				
	PE	C	A	E	Aq	PE	C	A	E	Aq	PE	C	A	E	Aq
Steroids	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-
Alkaloids	-	-	+	+	-	-	-	+	+	-	-	-	-	+	-
Phenolic compounds	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-
Flavonoids	+	-	+	+	+	-	-	+	+	-	-	-	+	+	-
Saponins	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+
Tannins	-	-	-	+	-	-	-	-	+	+	-	-	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Catechine	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-
Amino acids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PE: Petroleum Ether; C: Chloroform; A: Acetone; E: Ethanol; Aq: Aqueous

Among the various solvents used to extract the phytoconstituents in *C. gigantea*, ethanolic extracts possess the maximum percentage of phytocompounds 66.66% followed by acetone 50% and aqueous 41.66% extracts. The results documented the presence of phenolics, terpenoids and cardiac glycosides in all the five tested extracts of *C. gigantea*. Saponins determined their existence in three different extracts followed by steroids, alkaloids and tannins in two extracts. Coumarin was present only in ethanolic extract of *C. gigantea*.

Phytochemical studies on *C. crinita* revealed the presence of phenolics, saponins, tannins and terpenoids in three different extracts whereas steroids and flavonoids were present in two extracts. Alkaloids and cardiac glycosides confirmed their presence only in ethanolic extracts. Among the tested crude extracts, ethanolic extracts determined the presence of more frequency 58.33%) of phytoconstituents whereas the other extracts showed minimum number of compounds.

The cladogram constructed based on the results of preliminary phytochemical profile of studied *Cyathea* species showed two clades viz., C_1 and C_2. The clade C_1 was shared between *C. nilgirensis* and *C. gigantea* whereas clade C_2 showed the unique presence of *C. crinita* (Figure 1).

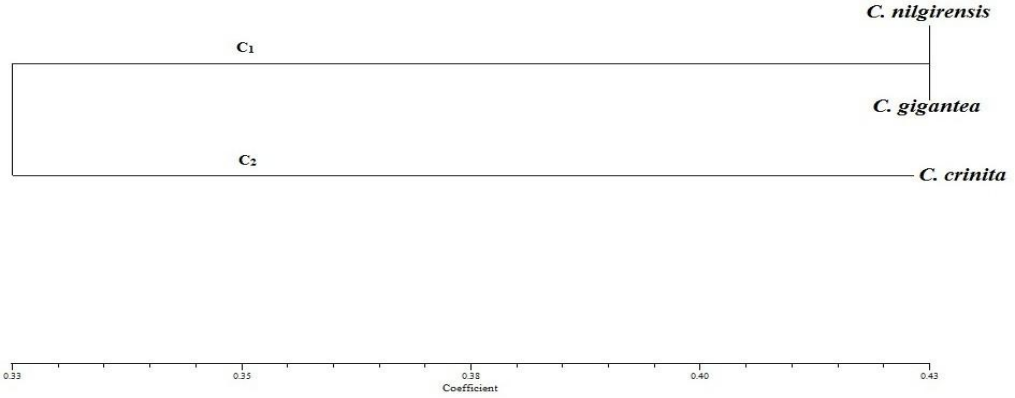

Figure 1. Cladogram based on qualitative phytochemical profile of *Cyathea* species.

The dry weight yield of different extracts of *C. nilgirensis, C. gigantea* and *C. crinita* were demonstrated in (Table 2). The results of extractive values provide a basis to identify the quality and purity of the plant material. Fluorescence analysis of *C. nilgirensis, C. gigantea* and *C. crinita* plant powders carried out under visible and UV light showed more or less similar characters (Figure 2). The fluorescence analysis revealed the similar and distinguished colour characteristics based on various chemical reagents and solvents employed.

Table 2. Dry weight yield for studied species of *Cyathea.*

Extracts	Extraction yield % (1 g)		
	C. nilgirensis	*C. gigantea*	*C. crinita*
Petroleum ether	0.15	0.27	0.21
Chloroform	0.28	0.31	0.58
Acetone	0.21	0.41	0.60
Ethanol	**0.57**	**0.76**	**0.85**
Aqueous	0.53	0.34	0.63

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Treatment	*C. nilgirensis*		*C. gigantea*		*C. crinita*	
	Ordinary light	UV light (365 nm)	Ordinary light	UV light (365 nm)	Ordinary light	UV light (365 nm)
Plant powder as such	Green	Brown	Light Green	Brown	Green	Brown
Petroleum ether extract	Dark Green	Reddish Brown	Dark Green	Brownish Green	Light Green	Brown
Chloroform extract	Light Green	Brown	Dark Green	Dark Red	Dark Green	Brownish Green
Acetone extract	Light Green	Brownish Green	Green	Brown	Light Yellow	Greenish Yellow
Ethanolic extract	Dark Green	Yellowish Green	Brown	Yellowish Green	Light Red	Dark Green
Aqueous extract	Green	Light Green	Brown	Brownish Green	Green	Brown
Powder + Con. H_2SO_4	Brown	Bluish Green	Dark Green	Brown	Brown	Brown
Powder + Con. HCl	Dark Green	Reddish Brown	Light Green	Dark Green	Brown	Brown
Powder + CH_3COOH	Yellowish Green	Pale Green	Light Green	Yellowish Green	Yellowish Green	Dark Green
Powder + NaOH	Light Yellow	Greenish Yellow	Green	Greenish Yellow	Light Green	Dark Green
Powder + 5% $FeCl_3$	Yellowish Green	Brown	Yellowish Green	Dark Green	Dark Green	Brown

Figure 2. Fluorescent characters of studied *Cyathea* species.

DISCUSSION

Phytochemical analysis was performed on whole plant extracts of *C. nilgirensis, C. gigantea* and *C. crinita* to reveal the presence of various active constituents which are known to exhibit medicinal as well as physiological activities. The results showed the presence of phytochemicals such as phenolics, flavonoids, tannins, cardiac glycosides, terpenoids, steroids, saponins and alkaloids. The presence or absence of the phytoconstituents in a particular species depends upon the organic solvent used for extraction and the physiological aspect of the selected species of *Cyathea*. In the present study, ethanolic extracts demonstrated the presence of maximum metabolites in *C. nilgirensis* (8/12), *C. gigantea* (8/12) and *C. crinita* (7/12) compared to other solvents employed.

Plant phenolic compounds include flavonoids, tannins, glycosides, coumarins, anthraquinones, lignans and lignins. They may act as phytoalexins, anti-feedants and attractants for pollinators. In addition, they act as contributors to the plant pigmentation [21]. Phenolics have also been considered powerful antioxidants *in vitro* and proved to be more potent than Vitamin C, E and carotenoids [22]. Phenolics are thought to provide a means of protection against UV-B damage and subsequent cell death by protecting DNA from dimerization and breakage [23]. Therefore, plants in high altitude areas which are exposed to a number of stress factors such as low air temperature, decreased partial O_2 pressure, increased UV radiation and unfavourable water regime have generally increased accumulation of antioxidants [24]. The studied three species of *Cyathea* collected from high altitude regions of Western Ghats, South India also showed high accumulation of phenolic compounds and the results of the present study coincided with previous observations.

Flavonoids including biflavonoids, homoflavonoids, flavone glycosides and flavonol glycosides are an important group of secondary metabolites represented in pteridophytes. Amentoflavone and ginkgetin flavonoids found in ferns exhibit neuroprotective activity against cytotoxic stress. This property suggests their possible use in the treatment of neurodegenerative diseases such as stroke and Alzheimer's disease [25]. They also exhibit a wide range of biological activities viz., antimicrobial, anti-inflammatory, anticarcinogenic, hepatoprotective, antithrombotic, anti-allergic and vasodilatory actions. Many of these biological functions have been attributed to free radical scavenging property of these compounds [26-28]. Tannins are good antimicrobial agents which precipitate protein thereby providing waterproof layer on the skin when used externally or protect the underlying layers of the skin and limit the loss of fluid [29]. In particular, the tannin containing remedies are in use as antihelmintics, antioxidants, cancer treatment and to chelate dietary iron [30-33]. Glycosides are known to lower the blood pressure [34].

Alkaloids rank among the most efficient and therapeutically significant plant metabolites [35]. They are one of the largest groups of phytochemicals in plants having significant effects on humans which have led to the development of powerful pain killer medications [36]. Plant alkaloids are used as basic medicinal agents for analgesic and antispasmodic activities [37]. They are also used as antidepressant (morphine), stimulants (caffeine), anaesthetic (cocaine), anti-tumour (vinblastine) and antimalarial (quinine) agents [38, 39].

Terpenoids are the main component of many plant essential oils [40]. They are a diverse group among the pteridophytes which includes triterpenoids, diterpenoids, hemiterpene glycosides and clerodane diterpene glycosides. Terpenoids are medicinally significant for a wide range of treatments viz., cytotoxic against human cancer cell lines and anti-inflammatory activity [41]. Steroids and saponins are the derivatives of terpenoids. Steroids may serve as an intermediate for the biosynthesis of downstream

secondary products and it is believed to be a biosynthetic precursor for cardenolides in plants. The presence of steroids in every organism suggests that they have a powerful role in chemosystematics [42, 43]. Saponins have a diverse range of medicinal properties viz., haemolytic, anti-inflammatory, anti-cancer, molluscicidal, insecticidal and antimicrobial [44-47]. Saponins are also of great interest as valuable adjuvants [48]. The results of qualitative phytochemical analysis confirmed the presence of phenolics, flavonoids, tannins, cardiac glycosides, terpenoids, steroids, saponins and alkaloids in the studied *Cyathea* species. The present study results suggest that the studied *Cyathea* species may be used as antioxidant, anticancer, antimicrobial, anti-inflammatory, insecticidal and haemolytic agents.

Talukdar et al carried out qualitative phytochemical analysis on *C. gigantea* and *Cyathea brunoniana* [49]. The results confirmed the presence of steroids, flavonoids and saponins in petroleum ether, ethyl acetate, acetone and methanolic extracts. Alkaloids and tannins were failed to show their presence in the tested extracts. In the present study, steroids, flavonoids, saponins, alkaloids and tannins were present in different extracts of *C. nilgirensis, C. gigantea* and *C. crinita.* The results of the present study were contrary to the previous observations. *Kiran et al* carried out preliminary phytochemical screening of *C. gigantea* and confirmed the presence of triterpenes, sterols, saponins and flavonoids. In the present study also, saponins and flavonoids were present in *C. gigantean*. Hence, the results were directly coincided with the previous observations. Hepatoprotective activity of *C. gigantea* was also confirmed by *Kiran et al*. [50]. The other pharmacological properties of *Cyathea* species were unexplored. The results of the present study paved a way to find the chemical constituents of the studied *Cyathea* species which may lead to quantitative estimation and also in locating the source of bioactive principles for various pharmacological properties.

The physico-chemical evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. Extractive values are useful for the determination of exhausted drugs and help in estimation of specific constituents soluble in a particular solvent [51]. Correct identification and quality assurance of the starting material is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy [52]. For fluorescence analysis, the powders of selected *Cyathea* species were treated with various chemical reagents. The fluorescence colour is unique for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. Similar to the present study, *Kala et al* previously applied fluorescence characters as a tool to characterize the different medicinal plants of South India. The results of the fluorescence analysis of studied *Cyathea* species may be applied to identify the purity of the drug in the pharmaceutical industries [53].

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