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Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantea* (Wall. Ex. Hook.) Haltt. and *Cyathea brunoniana*. Wall. ex. Hook. (Cl. & Bak.)

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Abstract

Cyathea gigantea and Cyathea brunoniana are the two available tree ferns of Southern Assam. Very few and sporadic works are there in the literature regarding the phytochemistry of both the plant. Qualitative phytochemical analysis reflects the presence of steroid, flavonoid, and saponin in both the plant extract. TLC profiling of all plant extracts gives an idea about the presence of various phytochemicals.Different Rf (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals.

Key words: *Cyathea gigantea*, *Cyathea brunoniana*, Phytochemical screening, TLC Profiling, & Retention factor(Rf.).

Introduction

"Tree Fern" is a somewhat arbitrary terms that has been applied to any fern with a large, erect rhizome, a portion of the fern that bears the leaves. Tree ferns are all true ferns in that they are flowerless plants and reproduce by the production of spores. The spores are developed in sporangia on the underside of the leaves or fronds.(Braggings et al., 2004).In India, members of Cyatheaceae family are widely distributed in Sikkim, Darjeeling Hills, Meghalaya, Assam, Khashi Hills, Arunachal Pradesh, South and Central India

In 2002, an article entitled "A plea for conservation of threatened tree fern (*Cyathea gigantea*)" was published by M.L. Khan *et al*., in which they expressed their concern that especially this tree fern is on the verge of being extinct in and around the Itanagar (Arunachal Pradesh). They noticed the total absence of young population, i.e. seedlings and saplings of *Cyathea* species in the aforesaid region. They analysed the prevailing situation, and pointed out the probable causes of such failure in regeneration of *Cyathea* species and suggested means of improving upon the situation. Though this observation was linked to Arunachal Pradesh, it brought to fore the necessity of taking care of the *Cyathea* species in general and *C. gigantea and C. brunoniana* in particular not only in Arunachal Pradesh but also in the whole of northeastern India including Assam. Motivated by this importance, the present work has been undertaken. Since in Southern Assam, two *Cyathea* species, viz. *Cyathea gigantea* and *Cyathea brunoniana* are available, it was felt timely and necessary to undertake a systematic study of the two aforesaid tree ferns which are endemic to this part of Assam.

Very little works has been done so far in genus *Cyathea*. The first investigation on flavonoid constituents in the genus *Cyathea* was carried out by Harada et al., (1955).They analyzed the leaves of *C. fauriei* and *C. hancockii* during a comprehensive survey on the distribution of flavones, flavonols and flavanones in Japanese ferns. Recently in 2008, Appel et al., in their paper

"Antioxidant and Hepatoprotective effects of *Cyathea phalerata* Mart. (Cyatheaceae)" reported the antioxidative potentiality of *Cyathea phalerata*.

Juneja *et al.*, (1990) isolated hentriacontane, â-sitostanone, diploterol, sitosterol, hop-29-ol and oleanolic acid from the hexane-soluble fraction of the tree fern, *C. gigantea*. So, It was the need of time to explore some of the species of this genus specially *Cyathea gigantea* and *Cyathea brunoniana* which are available in Southern part of Assam, India, for better upgradation of knowledge regarding the phytochemicals of this genus.

Materils and Method

Preparation of plant extracts

Each plant part of each species was extracted sequentially with petroleum ether, bp 40-60º (abbreviated as PE), ethyl acetate (EA), acetone and methanol by keeping the shade-dried, Powdered plant parts (500 g lot) dipped in the respective solvents (3 lit each) for three days. To clarify, the marc (i.e. plant material left after extraction with solvent, and then air-dried) left after extraction with PE was extracted with EA. The marc left thereafter was extracted with acetone, and finally, the marc left after extracted with methanol. This led to the production of four types of extracts, viz. petrol-soluble fraction (A), EA-soluble fraction (B), acetone-soluble fraction (C) and methanol-soluble fraction (D) from each of caudex and leaves. These extracts were labelled as A(S) – D(S) and A (L) – D (L), where the labels S and L indicate extracts of caudex and Leaves, respectively. A total of eight extracts for each of the two plant species was thus obtained which were examined chemically and screened for antimicrobial activities.

Protocol for preliminary phytochemical screening

The tests have been done to find the presence of the active chemical constituents such as alkaloids, steroids, flavonoids, reducing sugar and tannin by the following procedure.

Alkaloids

Alkaloids solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation. (Siddiqui and Ali,1997).

Steroid

20mg of extract was treated with 2.5 ml of acetic anhydride and 2.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids. (Siddiqui and Ali, 1997)

Flavonoid

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones. (Siddiqui and Ali, 1997).

Tannins

To 0.5 ml of extract solution 1ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

Reducing Sugar

To 0.5 ml of extracts solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

TLC Analysis of the Fractions:

Each of the aforesaid sixteen extracts was, to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silical gel (TLC-grade; Merck India). For each extract, five different solvent systems were used as developing sytems. These were PE-EA=9:1,

17:3, 4:1, PE-EA-M=18:1:1 and PE-EA-M=17:2:1 where the standard abbreviations used are: PE = petroleum ether, bp 40-60º; EA = ethyl acetate and M = methanol. In each case, the spots were visualised by exposure of the plates to iodine vapour.

Result

Result of Preliminary phytochemical screening:

Table 1: Qualitative phytochemical analysis of extracts of Caudex and Leaves of *Cyathea gigantea* in different solvent system

Extracts	Alkaloid	Reducing Sugar	Flavonoid	Steroid	Saponin	Tannin
A(S)	-	-	+	+	+	-
B(S)	-	-	+	+	+	-
C(S)	-	-	+	+	-	-
D(S)	-	-	+	+	+	-
A(L)	-	-	+	+	+	-
B(L)	-	-	+	+	+	-
C(L)	-	-	+	+	+	-
D(L)	-	-	+	+	-	-

(+), Presence. (-), Absence

Table 2: Qualitative phytochemical analysis of extracts of Caudex and Leaves of *Cyathea brunoniana* in different solvent system.

Extracts	Alkaloid	Reducing Sugar	Flavonoid	Steroid	Saponin	Tannin
A(S)	-	-	+	+	-	-
B(S)	-	-	+	+	+	-
C(S)	-	-	+	+	-	-
D(S)	-	-	+	+	-	-
A(L)	-	-	+	+	+	-
B(L)	-	-	+	+	+	-
C(L)	-	-	+	+	+	-
D(L)	-	-	+	+	+	-

(+), Presence. (-), Absence

Results of TLC analysis of the fractions

The retention factors (Rf) for each of the sixteen extracts in different solvent systems are detailed below.

Table 3:The retention factor (Rf)for each of the eight extracts of caudex and leaves of *Cyathea gigantea* in different solvent system**.**

Extracts	Developing solvent systems			
	PE-EA (9:1)	**PE-EA (17:3)**	**PE-EA (4:1)**	**PE-EA-M (18:1:1)**
A(S)	0.72,0.69,0.79	0.66, 0.84, 0.74	0.80,0.57,0.77	0.73,0.66, 0.78
B(S)	0.71,0.57,0.64	0.62,0.74,0.57	0.65, 0.75,0.80	0.71,0.63, 0.68
C(S)	0.65,0.72,0.58	0.54,0.71,0.67	0.74,0.49,0.68	0.64,0.55,0.71
D(S)	0.48,0.56,0.59	0.63,0.51,0.60	0.66, 0.58,0.53	0.59, 0.72,0.63
A(L)	0.75,0.67,0.70	0.80, 0.54, 0.74	0.70,0.67,0.77	0.73,0.76, 0.69
B(L)	0.70,0.50,0.64	0.62,0.76,0.47	0.82,0.68,0.72	0.78,0.60, 0.58
C(L)	0.60,0.68,0.49	0.54,0.71,0.60	0.66,0.49,0.68	0.64,0.59,0.69
D(L)	0.46,0.57,0.42	0.63,0.60, 0.55	0.66, 0.55,0.70	0.54, 0.72,0.69

Table 4: The retention factor (Rf) for each of the eight extracts of caudex and leaves of *Cyathea brunoniana* in different solvent system**.**

Extracts	Developing solvent systems			
	PE-EA (9:1)	**PE-EA (17:3)**	**PE-EA (4:1)**	**PE-EA-M (18:1:1)**
A(S)	0.60,0.70,0.67	0.67, 0.84, 0.74	0.79,0.84,0.75	0.80,0.69, 0.76
B(S)	0.70,0.67,0.51	0.62,0.70,0.77	0.66, 0.73,0.79	0.78,0.68, 0.59
C(S)	0.69,0.59,0.65	0.61,0.71,0.55	0.69,0.51,0.73	0.60,0.71,0.75
D(S)	0.58,0.56,0.47	0.59,0.51,0.72	0.56, 0.67,0.71	0.61, 0.75,0.65
A(L)	0.74,0.69,0.56	0.78, 0.71, 0.74	0.80,0.63, 0.77	0.82,0.76, 0.65
B(L)	0.67,0.50,0.59	0.67,0.76,0.52	0.79,0.62,0.70	0.77,0.64, 0.58
C(L)	0.62,0.68,0.49	0.52,0.74,0.56	0.69,0.70,0.54	0.74,0.58,0.61
D(L)	0.54,0.67,0.48	0.63,0.57,0.70	0.66, 0.59,0.70	0.69, 0.72,0.49

PLATE-1

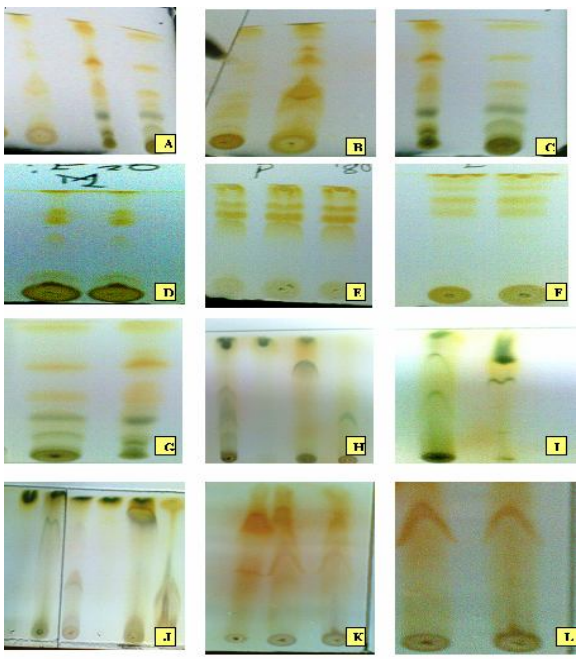

PLATE-1; TLC Profile of extracts. of *C. gigantea* **A.** Acetone extract of caudex at PE-EA- M (17:2:1) **B.** Acetone extract of caudex at PE-EA (9:1) **C**. Acetone extract of leaves at PE-EA (9:1) **D.** PET extracts of leaves at PE-EA (9:1) **E.** Acetone extract of leaves of at PE-EA (4:1) **F.** EA extract of caudex at PE-EA-M (17:2:1) **G.** EA extracts of Caudex at PE-M (9:1) **H-I.** Acetone extract of leaves at PE-EA- M (17:2:1) **J-K**. Methanol extract of leaves and caudex PE-EA- M (18:1:1)**L.** Methanol extract of caudex at PE-EA- M (18:1:1).

PLATE-2

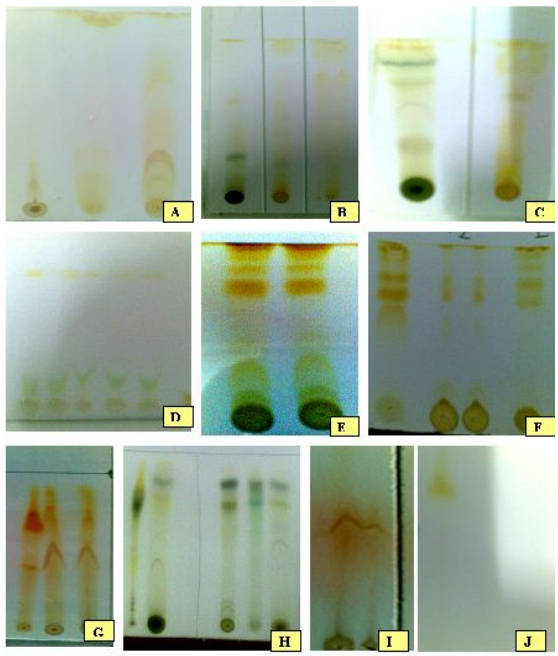

PLATE-2; TLC Profile of extracts. of *C.brunoniana* **A.** Acetone extract of caudex at PE-EA- M (18:1:1) **B.** Acetone extract of caudex at PE-EA (9:1) **C**. Acetone extract of leaves at PE-EA (9:1) **D.** PET extracts of leaves at PE-EA (9:1) **E.** Acetone extract of leaves of at PE-EA (4:1) **F.** EA extract of caudex at PE-EA-M (17:2:1) **G.** EA extracts of Caudex at PE-M (9:1) **H-I.** Acetone extract of leaves at PE-EA- M (17:2:1) **J.** Methanol extract of caudex at (17:2:1).

Discussion

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for all sixteen extracts showed significant indication about the presence of metabolites. Steroid and flavonoid were found to be present in the extracts of the leaves and caudex of both the tree ferns. While alkaloids, tannins and reducing sugar could not be detected in the extracts. Saponins were not uniformally found in both the cases. These findings of phytochemicals were good enough to reflect its importance. Both these plants can be used for further phytochemical analysis .TLC profiling of all 16 extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by Column Chromatography. Compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system.

In the present state of affairs, TLC profiling of all the plant extract of caudex and leaves in different solvent system indicated the presence of diverse type of phytochemicals in these plant. Different Rf values of the compound also reflects an idea about their polariy. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

Conclusion

Preliminary phytochemical analysis revealed the presence of steroids, flavonoids and saponins in all the 16 extracts of the caudex and leaves of *Cyathea gigantea* and *Cyathea brunoniana*. TLC profiling of plant extracts in different solvent system confirms the presence of diverse group of phytochemicals.

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