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Original Research Article

Pharmacognostical and Preliminary Phytochemical Screening of The Leaf *Phlogacanthus thyrsiformis* (Roxb. Ex Hardw) Mabb. (Acanthaceae)

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ABSTRACT

The aim of the study is to cover the pharmacognostical and preliminary phytochemical screening of the leaf *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. belonging to the family Acanthaceae is a natural taxon. Macroscopical and microscopical characters were studied. Physico-chemical parameters like total ash value acid insoluble ash value, water-soluble ash value and sulphated ash value were observed. Alcohol soluble extractive value, water-soluble extractive values were also observed and loss on drying was observed as %w/w. The foaming index was found. Preliminary phytochemical studies show the presence of important phytoconstituents. The presence of phytoconstituents explains that the plant must have valuable medicinal properties which must be explored.

Keyword: *Phlogacanthus thyrsiformis*; macroscopical and microscopical studies; phytochemical screening

INTRODUCTION

Phlogacanthus thyrsiformis (Roxb. ex Hardw) Mabb. belonging to the family Acanthaceae is an evergreen shrub upto 2.4 m high, branchlets quadrangular, leaves are found 13-35 cm long, oblanceolate, elliptic oblong, acute or acuminate, entire. Flowers are found in terminal elongated, thyrsoid panicles, up to 30 cm long. Capsule is 3.8 cm long, linear clavate. In early spring the plant becomes showy with its dense cylindrical spikes of brick red velvety flower. Calyx lobe is 6.8 mm, bristly haired. Bracts are 6 to 12 mm long. Seeds are disc like. Flowering are found in the month of February to April [1-3].

Phlogacanthus thyrsiflorus Nees is found usually in the sub tropical Himalayas from Ravi to Bhutan, Upper gangetic plain, Bihar, North

Bengal, plains and hills of Assam and Bangladesh [2, 3].

Leaves of *Phlogacanthus thyrsiflorus*, *Scutellaria discolor* and *Swertia chirata* 40 g each boiled in 2 litres water in a closed vessel and the steam so liberated exposed to abdominal portion. 5 ml of the concoction was also taken orally once a day for 3 days. Leaf of *P. thyrsiflorus*, *S. discolor* and tender shoot of *Arundo donax* in the ratio 40 g each boiled in 2 litres of water and the steam liberated exposed to all body parts for a week period. Irregular women menstrual problem [4]. Flower is used to treat ophthalmia. Bark is used to treat in acidity, indigestion, worm, heart disease [5].

In North Bengal, different community used *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. as Opthalmia, acidity, indigestion, cough and cold, whooping cough, asthma, chronic bronchitis, worm, heart disease, rheumatism, diarrhea, dysentery, piles, pox, antiseptic, leucoderma [6].

MATERIAL AND METHODS

Collection of the specimen

The plant species for the proposed study has been collected from Terai and Duars areas of West Bengal. Proper care was taken to select healthy plants with normal organs. The species for the proposed study was identified as *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. by Professor, Dr. A.P. Das, MSc, DIIT, PhD, FIAT, FNScT, Ex-Head. Department of Botany, Ex-Director. Centre for Life Sciences, Ex-Coordinator: Department of Biotechnology, Taxonomy & Environmental Biology Laboratory, Department of Botany, North Bengal University, Darjeeling 734 013, W.B., India and voucher specimen (Accession No. 09693) was deposited in the Herbarium of the same department.

Sectioning

The macroscopic characters of the leaf such as colour, odour, taste, nature, texture were studied for morphological investigation. For anatomical studies free hand sectioning was performed to obtain a thin transverse section of leaf with the help of 7 o'clock blade and central portion of mature leaflets were taken. The thick sections were stained with safranin.

Photomicrographies were taken with Nikon lab photo – microscopic unit. The quantitative microscopy was studies as per the procedure given by Wallis [7, 8]. The powder analysis has been carried out according to the method of Brain and Turner [9].

Descriptive terms of the anatomical features are taken from the standard Anatomy books [10-14].

Physichochemical parameters

The residue left after incineration of a drug is designated as ash. The residue originate from inorganic elements present in the plant is called as physiological ash. It varies with in definite limits according to types of soil, dust, sand and mineral impurities and admixture of other drugs may alter the ratio. Ash value represents the inorganic salts naturally occurring in the drug and adhering to it. Total ash is the residue remaining after incineration. The acid insoluble ash is the part of total ash which is insoluble in dilute hydrochloric acid. Mixing of sulphuric acid with powdered crude drug before ashing and this sulphated ash is normally less fusible than ordinary ash.

Extractive value which is an indicative of approximate measures of chemical constituents and nature of the constituents was performed using ethanol and distilled water as solvents.

The moisture content was determined in reference to air-dried sample by loss on drying method. Ash analysis, Extractive value and loss on drying were performed. .

Many plant (medicinal) materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts are measured in terms of ‘foaming index’.

All the procedure taken from the standard books [14-16].

Fluorescence analysis and behavioral change

A very small quantity of powdered drug was kept in a watch glass in an accumulated form. Then 2-3 drop of respective reagent was added and the fluorescence character of the plant powders was studied both in daylight and UV light as such and after treatment with the reagent like sodium hydroxide, picric acid, acetic acid, Hydrochloric acid, nitric acid, iodine, ferric chloride etc. similarly the fluorescence analysis of the plant extract was observed under visible and UV light. The behavior of the leaf powder in different chemical reagent also observed [17].

Extraction

Successive extraction was done in soxhlet extractor using the following solvents: Petroleum Ether, Ethyl Acetate, Methanol, and Water. All the extracts obtained by extraction were subjected to various qualitative tests for the identification of various plant constituents present in the species [14].

Thin layer chromatography

Ascending thin layer chromatography was performed for the separation of phytocontituents. As Ethyl acetated extract was showed more potent phytoconstituents and as Ethyl acetated extract gives flavonoids fractions and now a day flavonoids is an important potent phytoconstituents so it was subjected for this study with different ratios of various solvents, and it's showed clear isolation and resolution of spots in different solvent ratio on the TLC plates. Authentic flavonoid samples of Quericetin and Kaempferol also run in the TLC plates. Different spots developed in each solvent system were identified through UV light (510 nm) and the Rf values were accordingly calculated [18-20].

RESULTS

In macroscopical studies of the leaf (Fig. 1), it were found that it's shape was oblanceolate, elliptic-oblong, apex was acute or acuminate, margin entire, size was 7-10cm broad and 15-30 cm in length, colour was dark glossy green (upper surface), light green (lower surface), upper surface was smooth and lower surface was slightly rough, odour was aromatic, taste was pungent.

Fig. 1: Leaf of *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. (Acanthaceae)

In microscopy T.S of leaf shows Upper and lower epidermis with straight or slightly wavy anticlinal walls. Palisade parenchyma which are present just below the adaxial epidermis and spongy parenchyma which are below the palisade cells. Xylem and Phloem are found surrounded by the prominent endodermis layer. Few collenchymas cell are present below upper and above lower epidermis (Fig. 2).

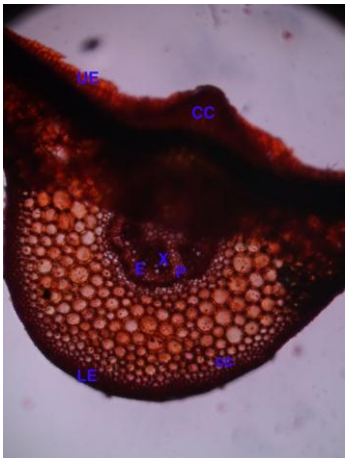

Figure 2: Microscopy of the leaf of *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb.

UE: Upper epidermis, LE: Lower epidermis, CC: Collenchyma, X: Xylem, P: Phloem, En: Endodermis

Physico-chemical parameters like total ash value (12.33 % w/w), acid insoluble ash value (3 %w/w), water-soluble ash value (0.66 %w/w) and sulphated ash value (9.3 %w/w) were observed. Alcohol soluble extractive value (0.60 %w/w), water-soluble extractive value (10.4 %w/w) were also observed and loss on drying was observed as 6.6 %w/w. The foaming index was found to be 111.11. The result of different physicochemical parameters are given in Table 1.

Table 1: Physico-chemical parameters of powdered of different plant materials

SL No.	Parameters	% w/w
1	Alcohol Soluble Extractive	0.60
2	Water soluble Extractive	10.4
3	Ash values	
	(i) Total Ash	12.33
	(ii) Acid Insoluble Ash	3
	(iii) Water Soluble Ash	0.66
	(iv) Sulphated Ash	9.3
4	Loss on Drying	6.6

The dried leaf of *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. were extracted with petroleum ether, ethyl acetate, methanol and distilled water by continuous hot soxhlet extraction and the percentage yield were found to be 0.20 % w/w, 1.65%w/w, 0.68% w/w and 1.62 % w/w respectively.

The fluorescence analysis and behavior of the leaf powder revealed wide range of fluorescence colours. In comparison, it was observed that all the parts showed similar colour ranges with mild differences. This may be due to the presence of similar phytoconstituents. The result of fluorescence analysis and behavior of the leaf powder are given in the Table 2 and 3.

Table 2: The fluorescence analysis of the powder of different plant materials

SL No.	Treatment with chemical reagents	Observation
1.	Powder as such	Deep Green
2.	Powder + 1N Sodium hydroxide in methanol	Yellowish Green
3.	Powder + 1N Sodium hydroxide in water	Pale Green
4.	Powder + 50% Hydrochloric acid	Deep Green
5.	Powder + 50% Sulphuric acid	Dark Black
6	Powder + 50% Nitric acid	Greenish Black
7	Powder + Petroleum ether	Colourless
8	Powder + Chloroform	Blackish Green
9	Powder + Picric acid	Greenish Black
10	Powder + 5% Ferric chloride solution	Deep Green
11	Powder + 5% Iodine solution	Bluish Green
12	Powder + Methanol	Pale Blue
13	Powder + (Nitric acid + Ammonia)	Bluish Green
14	Powder + Glacial acetic acid	Deep Blue
15	Powder + 5% Potassium Hydroxide solution	Black

Table 3: The behavior of the leaf powder of different plant materials when treated with different chemical reagents

SL No.	Treatment with chemical reagents	Observation
1.	Powder as such	Deep Green
2.	Concentrated Hydrochloric acid	Pale Green
3.	Concentrated Sulphuric acid	Dark Brown
4.	Concentrated Nitric acid	Light Brown
5.	Glacial acetic acid	Green
6	5% Sodium hydroxide solution	Pale Yellow
7	5% Potassium hydroxide solution	Pale Yellow
8	5% Ferric chloride solution	Orange
9	Picric acid	Yellowish Green
10	Ammonia	Light Green
11	1N Sodium hydroxide in methanol	Green
12	Powder + 1N Sodium hydroxide in Water	Pale Green

The extracts obtained were subjected to qualitative phytochemical tests to find out the active constituents, which showed presence of carbohydrates, fixed oils and fats, tannins and phenolic compounds, flavonoids and coumarins in petroleum ether extract, fixed oils and fats, tannins and phenolic compounds, flavonoids and coumarins in ethyl acetate extract, gums and mucilage, saponins, tannins and phenolic compounds, flavonoids and coumarins in

methanolic extract, proteins and amino acids, fixed oils and fats, alkaloids, saponins, tannins and phenolic compounds, flavonoids, lignin and coumarins in aqueous extract. Details of these tests performed to make a qualitative phytochemical analysis, are given in the Table 4.

Table 4: Qualitative phytochemical analysis of various extracts of different plant materials

Plant constituents	P.T. Extract			
	Pet. Ether	Ethyl Acetate	Methanolic	Aqueous
Carbohydrates	+	-	-	-
Proteins and Amino acids	-	-	-	+
Fixed Oils and Fats	+	+	-	+
Gums and Mucilage	-	-	+	-
Alkaloids	-	-	-	+
Glycosides	-	-	-	-
Steroids /Phytosterol	-	-	-	-
Saponins	-	-	+	+
Tannins and Phenolic compounds	+	+	+	+
Flavonoids	+	+	+	+
Triterpenoides	-	-	-	-
Lignin	-	-	-	+
Coumarins	+	+	+	+

(+) : Present, (-) : Absent, P.E.: Pet Ether, E.A.: Ethyl Acetate, M.: Methanol, A.: Aqueous

Ethyl acetate extract in n-BuOH: HOAc: H_2O (125:72:3) solvent system showed two spots with Rf values 0.97 (Florescence Yellow), 0.77(Orange). The spots are identified as Quericetin and Kaempferol as flavonoid.

DISCUSSION

In macroscopical and microscopical studies of the leaf characters are very common in leaves. The extracts obtained were subjected to qualitative phytochemical tests to find out the active constituents, which showed presence of carbohydrates, fixed oils and fats, tannins and phenolic compounds, flavonoids and coumarins etc in different extracts. So the leave part is very much rich in different phytoconstituents. On the basis of standard sample Rf values Quericetin and Kaempferol have been identified. Several other solvent system such as CH_2Cl_2 : HOAc: H_2O (2: 1: 1), n-BuOH: HOAc: H_2O (4:1:5), EtOAc: EtOH: HCOOH: H_2O (100:11:11:26), EtOAc: HCOOH: HOAc: H_2O (100:11:11:26), C_6H_6 : HOAc: H_2O (125:72:3) were also tried, but the solvent system containing n-BuOH: HOAc (125:72:3) gave best result.

CONCLUSION

The current study resulted in isolation compound Quericetin and Kaempferol (flavonol) from the Ethyl acetate extract of *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. presence of this constituent may be one of the contributing factors responsible for the different types of therapeutic activity. Further investigations are required to study the mechanism of actions of *Phlogacanthus thyrsiformis* (Roxb. ex Hardw) Mabb. and its constituents by which they exert their therapeutic effects.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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