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Research Article

Phytochemical screening and antioxidant activity of leaf extract of *Phlogacanthus thyrsiflorus* Nees. – a medicinal plant of Assam, India

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Abstract

Medicinal plants are the richest bioresource of drugs of both traditional and modern system of medicines and allied fields. One of the commonly used plant in traditional medicinal in Assam is *Phlogacanthus thyrsiflorus* Nees. belonging to the family Acanthaceae. Various parts of the plant have been used in preparation of folk medicines to treat fever, skin diseases, jaundice, indigestion, cough and cold, etc. The healing properties of the plant are due to the presence of certain phytochemicals and antioxidant property. These phytochemicals are non-nutritive plant components that have either defensive or protective properties, whereas antioxidants are those compounds which terminate the attack of reactive species and reduce the risk of diseases. The present study is carried out to investigate the qualitative phytochemical constituents and antioxidant property of the plant. The antioxidant activity is evaluated by free radical scavenging activity of DPPH (1, 1-diphenyl-2-picryl-hydrazyl) method. The bioactive compounds from the crude methanolic extract of the plant are confirmed by LC-HRMS. The free radical scavenging activities were investigated based on the presence of phenols, flavonoids and tannins in the plant materials. The plant could be beneficial in modern synthetic drug formulation by virtue of the presence of antioxidant activity and significant bioactive compounds.

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of either traditional or modern systems of medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for designing synthetic drugs (Ncube *et al.*,2008). Plant-derived substances have recently become of great interest owing to their versatile applications. Phytochemicals with nutraceuticals properties present in food are of enormous significance due to their beneficial effects on human health (Dixon and Strack, 2003).Most of the best sources of plant medicines are the aggregates of their respective secondary metabolites that are biosynthesized by the plants. One such commonly used plant in preparation of folk medicine in Assam is *Phlogacanthus thyrsiflorus* Nees. The various parts of the plant has been reported to be used as components in several folk medicines to treat fever, antidote to pox, skin diseases like sore, scabies, jaundice, liver and spleen diseases, indigestion, acidity, gastritis, pharyngitis, chronic leucorrhoea, cough and cold, chronic bronchitis, asthma and rheumatism (Jaiswal, 2010; Ali *et al.*, 2012; Paharia *et al.*, 2013).

The healing properties of the medicinal plants are due to the phytochemicals present in them. These phytochemicals work with nutrients and fibres to form an integrated part of defence system against various diseases and stress

conditions (Singh, 2005). The information on the plant signifies the enormous use of the plant, but only a small percentage has been investigated for its phytochemical screening. Presence of some preliminary phytochemicals also gives the hint to presence of some antioxidant activity of the plant material. Hence, the present study was aimed at screening phytochemicals and antioxidant properties of *P. thyrsiflorus.*

MATERIALS AND METHOD

Phlogacanthus thyrsiflorus Nees., commonly known as '*Rangabahaka*' or '*Teeta phool*'(in Assamese) is a gregarious shrub belonging to the family Acanthaceae (Anderson, 1867). It reaches to a height of 2.4 m with branchlets quadrangular, leaves 13-35 cm long, oblanceolate, elliptic-oblong, acute or acuminate and entire along the margin. Flowers are terminal, elongated, thyrsoid panicles, up to 30cm long; corolla tubular, curved; orange or brick-red in colour. Capsule 3.8 cm long linear-clavate (Tamang *et al*., 2005). For the present investigation, mature leaves of the plant were collected from the Botanical Garden, Gauhati University, Assam India.

Fig:1. *Phlogacanthus thyrsiflorus* Nees. (A). Habit (B). Inflorescence.

Preparation of plant extracts using suitable solvents

The freshly collected leaves were washed under running tap water to remove dirt and shade dried for 45 days. Finally, the dried leaves were ground into fine powder. 20gm of powdered plant material was soaked into 200 ml organic solvents *viz.*, water, ethanol and methanol for 24 hrs in an orbital shaker at 150 rpm in 30°C. The extracts were filter through the Whatman No. 1 filter paper. The extract was then allowed to evaporate. The condensed extracts were stored in airtight container at 4°C till further investigation (Tanti *et al*., 2010).

Preliminary phytochemical screening

The extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents using standard prescribed methods (Harborne 1984; Trease and Evans 1987; Edeoga *et al*., 2005).

For, alkaloid test, 2ml of each extract was acidified with a few drops of dilute hydrochloric acid and filtered followed by addition of 1ml of Mayer's reagent to it. The appearance of yellow colour precipitate indicates the presence of alkaloid. For test of carbohydrate, plant extracts were dissolved in 5ml distilled water and filtered. The filtrate was used to test (Fehling's test) for the presence of carbohydrates. For estimation of glycosides, a few drops of glacial acetic acid and ferric chloride were added to1 ml of each extracts. 3-4 drops of sulphuric acid were then added to it. Appearance of blue-green colour indicates the presence of glycosides. For testing the presence of saponins, 0.5 ml of each extract was shaken with 2ml of distilled water. Persistence of foam for ten minutes indicates the presence of saponins. For polysterols estimation **e**xtracts were treated with chloroform and the filtrate was treated with few drops of conc. Sulphuric acid and allowed to stand for a while. Appearance of golden yellow colour indicates the presence of diterpenes (Salkowski's test).

For estimation of phenols extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence phenols. For test of tannins extracts were treated with few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins. For flavonoids estimation, 2 ml of extract was treated with 5ml of dilute ammonia followed by addition of few drops of sulphuric acid. Yellow colour formation occurs. Upon further standing yellow colour disappear. For diterpenes estimation extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes. Lastly, for estimation of coumarins10% NaOH was added to the extracts and chloroform was added. Appearance of yellow colour indicates the presence of coumarins.

DPPH antioxidant scavenging

The free radical scavenging activity was measured by the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and Shui (2002). DPPH solution of 0.1 mM was prepared freshly in methanol and kept away light and then from the initial absorbance was measured at 517 nm using Spectrophotometer (Beckman Coulter, DU730). Final concentration of standard ascorbic acid and plant extracts were made at various concentrations (7.5µg, 15µg, 22.5µg, 30 µg) and then taken and final volume is adjusted to 10ml with methanol. The final volume were adjusted to 1ml by adding 1.25µl of methanolic extract and 375 µl of methanol in an eppendorf tube of 1ml and then placed in dark for 30mins at 27ºC. Methanol was used as blank and the experiment was expressed as the inhibition percentage (%) of free radical by the sample and was calculated as the formula follows:

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs control is the absorbance of DPPH + methanol
Abs sample is the absorbance of DPPH radical + sample (*i.e.* extract or standard)

LC-HRMS analysis

LC–HRMS was used for identification of few major bioactive compounds from the crude methanolic extract of *Phlogacanthus thyrsiflorus* (Model-Agilent Mass Hunter Z400) system. The analytical column was maintained at 40°C, while the sample manager at 15°C. The mobile phases used were (a) Ultrapure water/methanol/formic acid 9/1/0.1 and (b) Methanol acidified with 0.1% formic acid. Separation was achieved using the mobile phase gradient as 100% A for 1 min, from 0% to 10% of phase B in 0.1 min, linear gradient to 15% B in 4 min, linear gradient to 50% B in 1.8 min, to 70% B in 1.7, to 80% B in 1.1 min, to 100% in 1 min held for 3.5 min. The injection volume was 10 µl and the flow rate was set at 0.4 ml min−1. The chromatographic run time was of 14.5 min per analysis. Ionization parameters were optimized with direct infusion of each substance, including IS methamphetamine-D5, by an external syringe at 10 µl min^{-1}.The chosen tune for the heated electrospray ionization (HESI) source had the following settings: source current at 5 µA, sheath gas and auxiliary gas (either nitrogen) flow rates at 35 and 18 arbitrary units, respectively; capillary temperature at 290°C, the capillary voltage at 45 V, the tube lens voltage at 90 V, the skimmer voltage at 22 V.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Phytochemical studies done on different plant extracts of *P. thyrsiflorus* using various solvents showed different reactivity due to their difference in solvent polarity. Water extract showed the presence of alkaloids, saponins, phytosterols, phenols, tannins, flavonoids, coumarins, and diterpenes. Ethanol extracts showed the presence of saponins, phenols, tannins, coumarins, whereas methanolic extracts showed presence of saponins, phenols, tannins, and diterpenes (Table: 1).

Phytochemical screening was performed to identify phytochemicals in the ethanol, methanol, and water extracts of plant leaves. The qualitative screening of powdered crude drugs for their active ingredients was carried out using the following standard procedures (Trease and Evans, 1983; Mukherjee, 2002; Horborne, 2005).

Table: 1. Phytochemical screening of *Phlogacanthus thyrsiflorus*

Name of the Phytochemicals	Extract used		
	Water	Ethanol	Methanol
Alkaloids	+	–	–
Carbohydrates	–	–	–
Glycosides	–	–	–
Saponins	++	+	+
Phenols	++	+	+
Tannins	++	+	+
Flavonoids	+	–	–
Diterpenes	++	+	+
Coumarins	+	+	+
Polysterols	+	–	–

(++ Strong Reactivity, + Moderate Reactivity, -Not Detected)

DPPH radical scavenging activity

In the DPPH radical scavenging assay, antioxidants react with DPPH, and convert it to yellow coloured 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the radical-scavenging activity. In this test, *P. thyrsiflorus* extract exhibited a considerable antioxidant activity but more than the standard ascorbic acid. The antioxidant activity of ascorbic acid is highest with 58.76% DPPH scavenging at 30µg m/l concentration and IC50 value of 25.4µg/ml (Table: 2).

***In-vitro* antioxidant property analysis**

It was observed that the methanolic extract of *P. thyrsiflorus* have demonstrated dose dependent increase in the DPPH radical scavenging activity. Ascorbic acid (Standard) has shown IC50 at 25.4µg/ml concentration obtained by equation (Y = 8.279*X + 28.9) whereas MEPT has shown IC50 at 79.049 µg/ml concentration obtained by equation (Y = 6.297*X + 0.365) (Table 3; Fig. 2).

Table: 2. Analysis of DPPH radical scavenging

Concentration (µg/ml)	Scavenging (%)	
	Ascorbic acid	MEPT
7.5	33.89±3.66	6.66±2.22
15	48.89±0.98	6.66±2.22
22.5	57.06±3.52	21.48±2.56
30	58.76±1.95	24.44±4.44

Table: 3. Concentration of extract at DPPH radical scavenging activity 50% (IC_{50})

Sample extract	DPPH IC_{50} (µg/ml)
Ascorbic acid	25.48
***P. thyrsiflorus* extract**	79. 04

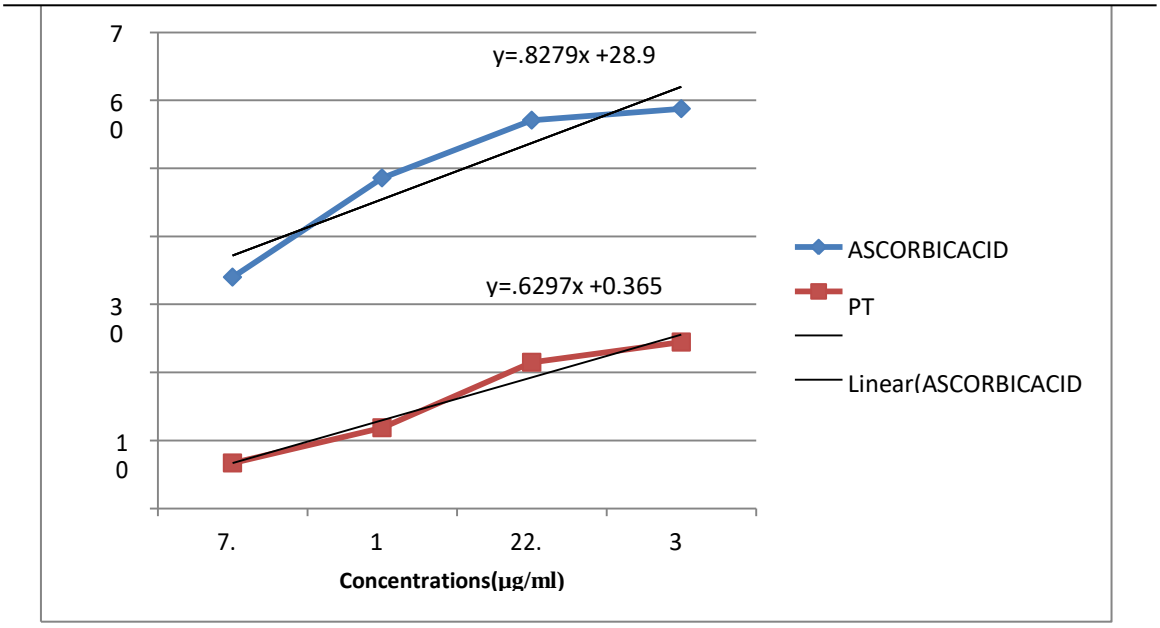

Fig: 2. DPPH radical scavenging activity (%) versus *P. thyrsiflorus* concentration (µg/mL). LC-HRMS analysis

HRMS analysis of methanolic leaf crude extract of *P. thyrsiflorus* showed the presence of novel compounds as presented in Table 4. To perform the online identification of each compound, LC gradients were developed to better separate solutes. Mass spectrometry is a powerful tool which provides structural information on molecules. However, ionization parameters have to be appropriate to the physicochemical properties of the compounds analyzed. The type of ionization source and mode of ionization were therefore optimized so as to be adapted to extract polarity. The electrospray ionization source (ESI) is well suited to polar compound. Thus, ESI was used to analyze the polar molecules present in the methanolic extract. However, in order to obtain the most exhaustive fingerprint possible, generic ionization parameters were applied in order to detect the majority of compounds properly. Here, only positive mode was tested. Then, high resolution mass spectrometry gave access to the accurate mass of compounds. The corresponding molecular formula was established with Data Analysis software and probable structures were assigned by searching in the Chemspider database. Finally fragmentation analysis confirmed the proposed identifications. To characterize the methanolic extract, the positive ionization mode was more appropriate for flavonoids detection. Molecules were detected mainly as deprotonated molecule ions $[M+ H]^+$ (Fig: 3).

Plants are important source of herbal medicines and in the recent era there has been a marked shift to herbal treatment of diseases due to the pronounced cumulative and irreversible effect of modern drugs. Keeping this in view the investigation was carried out on phytochemical investigation followed by antioxidant property of the leaf of *Phlogacanthus thyrsiflorus*. In this study the phytochemical analysis of the water, methanol and ethanol extracts of *P. thyrsiflorus* showed the presence of different group of secondary metabolites such as alkaloids, saponins, phenols, tannins and diterpenoids which are of medicinal importance. Though HRMS analysis, individual components were identified by comparison of their m/z values in the Total Ion Count(TIC) profile with those of the selected compounds describe in literature or by matching their MS/MS spectra with those. Identification of major compounds as Aspidocarpine, Ajmaline, Harmine, Hydroquinidine, Bilobalide, 1-naphthalenecarboxaldehyde, Sparteine, L-Histidine and Terbutylazine-2-hydroxy done by matching its tandem mass spectra with that of Mass Bank which confirmed *P. thyrsiflorus* as highly medicinal plant with antioxidant activities. Presence of flavonoids and derivatives at significant level showed concordance with the findings of antioxidant assay from the crude methanol extract of leaf of *P.*

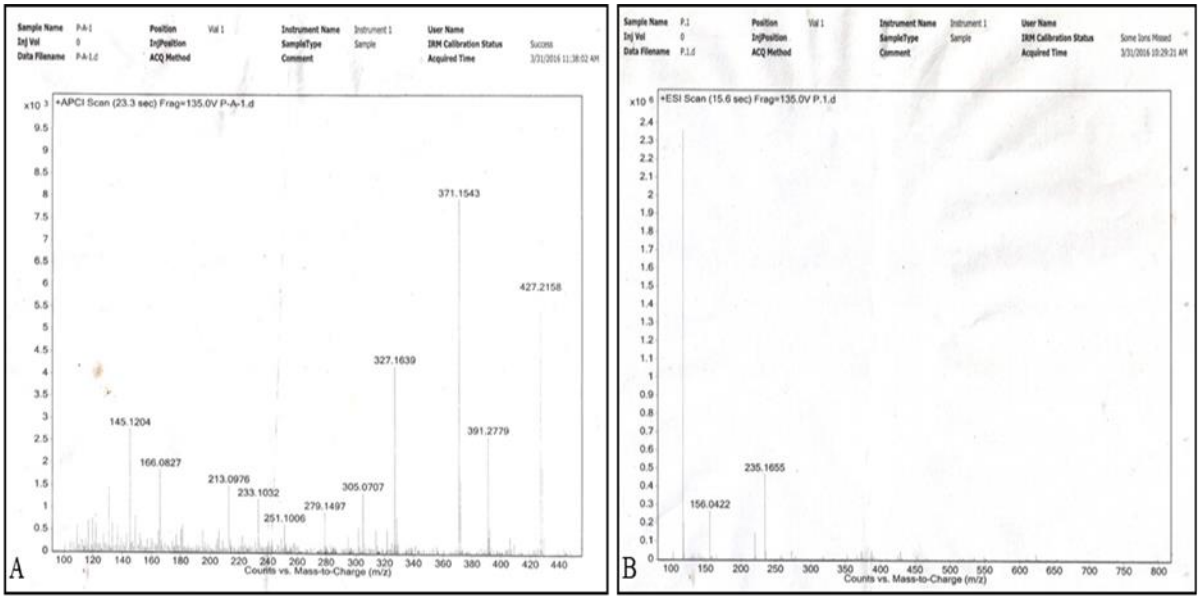

Fig: 3. HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH+ isotopic, A. spectra ranging from 100 – 440 m/z and B. 100 – 800 m/z.

Table: 4. Metabolites, calculated and their detected m/z ratio.

Metabolite	Ion Type	Calculated	Detected
1-naphthalenecarboxaldehyde, 2-hydroxy	[M+H]+	172.0524	145.1204
Harmine	[M+H]+	212.094963	213.0976
(-)-Bilobalide	[M+H]+	326.100168	327.1639
Hydroquinidine	[M+H]+	326.199428	327.1639
Aspidocarpine	[M+H]+	370.225643	371.15443
Ajmaline/Aritmina	[M+H]+	326.199428	371.15443
Sparteine	[M+H]+	234.209599	235.1655
L-Histidine	[M+H]+	155.06948	235.1655
Terbutylazine-2-hydroxy	[M+H]+	211.1433	156.0422

thyrsiflorus. Methanol extract of *P. thyrsiflorus* were subjected to screening for their possible antioxidant activities using DPPH and ascorbic acid assay methods at different concentration. Antioxidant activities of methanolic extract of *P. thyrsiflorus* showed lower antioxidant activity in DPPH method as compared to standard ascorbic acid. In this study, the antioxidant activity is determined by the DPPH assay of methanolic extract of the plant. The antioxidant property of *P. thyrsiflorus* shows the highest inhibition of 24.44% with IC50 value of 79.04 µg/ml. The present study showed that this plant can be considered as a good source of natural antioxidant and should be further analyzed for their chemical and biological properties.

The medicinal plants are used for discovering and screening of phytochemical constituents which are very helpful for manufacturing of new drugs. The phytochemical analysis is also very important for commercial interest in both research and pharmaceuticals companies for the manufacturing of drugs for different diseases. With this study it could be concluded that the phytochemical and antioxidant assay of *P. thyrsiflorus* would give valuable information for further characterization and exploitation of this important medicinal plant.

REFERENCES

Ali MS, 2012. Phytochemical Investigation of Stem bark of *Phlogacanthus thyrsiflorus* (Roxb) Nees. *Asian J Chemistry*, **24**(2): 784-786.

Anderson T, 1867. An enumeration of the Indian species of Acanthaceae. *J. Linn. Soc. Bot*, **9**: 425–526.

Dixon RA, and Strack D, 2003. Phytochemistry meets genome analysis and beyond. *Phytochemistry,* **62**(6): 815-816.

Edeoga HO, Okwu DE and Mbacbie BO, 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afri J Biotech*, **4**(7): 685-688.

Eloff JN, 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants. *Journal of Ethnopharmacology,* **60**: 1–8.

Harborne JB,1998. Phytochemical methods, Chapman Hall, London, pp 100 - 101.

Jaiswal V, 2010. Culture and ethnobotany of Jaintia tribal community of Meghalaya, Northeast India mini review. *Indian J of Trad. Knowledge,* **9**: 38.

Leong L and Shui G, 2002. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, **76**: 69-75.

Mukherjee A, Chaliha M and Das S, 2009. Study of analgesic activity of ethanol extract of *Phlogacanthus thyrsiflorus* on experimental animal models. *Bangladesh J Pharmacol*, **4**: 147-149.

Ncube NS, Afolayan AJ and Okoh AI, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methodsand future trends. *African Journal of Biotechnology*, **7** (12): 1797-1806.

Paharia AK and Pandurangan A, 2013. Evaluation of anti-antiasthmatic activity of hydroalcoholic extract of *Phlogacanthus thyrsiflorus Nees* in experimental animal. *The Global Journal of Pharmaceutical Research,* **2**(2): 1818-1824.

Singh AP, 2005. Promising phytochemicals from Indian medicinal plants. *Ethnobotanical leaflets*, **9**:15-23.

Tamang JP, Thapa MP, Sharma RM, Rai AK, Rai P and Dhaka lR, 2005. Carrying capacity study of Teesta Basin in Sikkim. *Biological Environment Food Resource,* **8**.

Tanti B, Buragohin AK, Gurung L, Kakati D, Das AK and Borah SP, 2010. Assesment of antimicrobial and antioxidant activity of *Dendrocnide sinuate* (Blume) Chew leaves- A medicinal plant used by ethnic communities of North East India. *Indian J Nat Prod Resour*, **1**(1): 17-21.

Trease E and Evans WC, 1987, Pharmacognosy, Billiare Tindall, London.

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