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Research Article

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Study of Secondary Metabolites of *Aristolochia roxburghiana* Klotzsch in Assam, India

B Dutta[1*] and K Gogoi[2]

[1]*Department of Botany, Sibsagar Girls College, Sivasagar, Assam, India*
[2]*Biotech Hub, Sibsagar Girls College, Sivasagar, Assam, India*

ABSTRACT

The present study was aimed to evaluate the presence of major secondary metabolites as well as to determine the total phenols, flavonoids and alkaloids contents in Aristolochia roxburghiana Klotzsch, an ethnomedicinal plant of Assam, India. Methanol extracts of leaves, stem and roots contain all studied compounds. The total alkaloid content (range from 220 to 400 mg/g) was found to be higher in comparison to phenols (ranging 4.48 to 16.65 mg/g) and flavonoids (2.19 to 6.68 mg/g), which is impressive and indicates the potential of the plant species to act as sources of wide range of drugs.

Keywords: *Aristolochia roxburghiana*; Ethnomedicinal plants; Phenols; Flavonoids; Alkaloids

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INTRODUCTION

Secondary metabolites, a group of organic substances comprising compounds like alkaloids, terpenoids, phenols, flavonoids, tannins, saponins, etc., having wide range of pharmacological activities, are produced through secondary metabolism in different plants. Phytochemical analysis of ethnomedicinal plants has been increased dramatically in the last few years owing to its relevance of the discovery of therapeutic agents and providing clues for less known plants or for new sources of bioactive compounds. *Aristolochia roxburghiana* Klotzsch, locally known as 'Nilokantha', belonging to family Aristolochiaceae, is a shrubby twining plant found in Assam; India. The plant has several ethno-medicinal applications. A paste, prepared from the roots, is kept in mouth for few minutes in tonsillitis. A decoction prepared from the whole plant (50 g) in about one liter of water, is given orally twice a day in malaria fever. Leaf-paste is applied locally in insect stings. Leaf-infusion is given orally in intermittent fever. Root is used as antidote to snake bite [1]. Thus, there is an ample scope to explore the pharmacological potential of the plant species. However, information regarding the phytochemical analysis of the plant species has been found to be limited. Thus, with an objective to evaluate the presence of major phytoconstituents, *viz.*, phenols, flavonoids, alkaloids, tannins, saponins as well as to determine the total alkaloids, flavonoids and phenols contents in leaves, stem and root extracts of *Aristolochia roxburghiana*, the present study has been conducted (Figures 1 and 2).

MATERIALS AND METHODS

Collection of Plant Material

Leaves, stems and roots of the plant are obtained freshly from the homestead garden. Plant species was identified with the help of regional floras and monographs [2-4]. Voucher specimen (Accession No. B.D. 1045) is submitted in the Herbarium of Sibsagar Girls College, Sivasagar, Assam and India.

Preparation of Extract of Plant Material

The plant materials (leaves, stem and roots) were washed with running tap water and air dried under shade. The complete drying materials were grained in mixer. Plant extracts were prepared using methanol as extracting

solvent.

Phytochemical Screening for Secondary Metabolites
Chemical tests were carried out qualitatively on the extract following methodologies adopted by Dutta [5] with slight modifications.

Test for Alkaloids

Dragendroff's test:
To test for presence of alkaloids 1 ml of each extracts were taken in three different test tubes. Few drops Dragendroff's reagent was added to the extracts and mixed it well. Formation of orange colour confirmed the presence of alkaloids.

Mayer's test:
2 ml of Mayer's reagent was added to each test tubes containing 1 ml of each extracts. Presence of alkaloids was confirmed with formation of a dull white precipitate.

Test for Flavonoids

Alkaline reagent test:
To test the presence of flavonoids, a few drops of sodium hydroxide solution were mixed with 1ml of each extracts. Intense yellow color was formed which become colourless after addition of few drops of dilute acetic acid and this confirmed the presence of flavonoids.

Shinoda test:
To 1 ml of each extracts few drops of concentrated HCl and a few pieces of magnesium turning were added. Presence of flavonoids in the plant sample was indicated by appearance of pink or magneta red colour.

Test for Phenolic Compounds
Ferric chloride test:
To test the presence of phenolic compounds few drops of ferric chloride solution were added to 1 ml of each extracts. Presence of phenolic compounds was well confirmed by formation of dark green color.

Test for Tannins
Lead acetate test:
To 1 ml of each extracts, a few drops of 10% lead acetate were added to check the presence of tannins. Appearance of precipitation confirmed the presence of tannins.

Test for Terpenoids
Salkowski's test:
To evaluate the presence of terpenoids first of all methanol extracts of leaves were mixed with chloroform and filtered. Formation of red color in lower layer suggests the presence of steroids. Presence of terpenoids was well confirmed by appearance of reddish brown colour of edges after addition of concentrated sulphuric acid.

Test for Saponins
Foam test:
1 ml of each extracts were diluted by adding 5 ml of distilled water and mixed it well. A few drops of olive oil were added to the extracts. Appearance of stable foam was considered as an indication for presence of saponins.

Determination of Total Phenol, Flavonoidand Alkaloid Contents
Folin-Ciocalteu method, as described by Dutta [5] with slight modification, was used for phenol content determination. 100 mg plant sample was dissolved in 10 ml methanol of 50% (v/v with distilled water). The solution was filtered. 0.5 ml of the filtrate was mixed with 2 ml of Folin-Ciocalteu reagent (1:1 diluted with distilled water) and mixed thoroughly. After five minutes 2 ml of 10% Na_2CO_3 solution was added. The solution was warmed for one minute, and then cooled. After one hour at room temperature absorbance was measured at 760 nm with UV-Visible spectrophotometer. Sample blank was concomitantly prepared containing 0.5 ml distilled water, 2 ml of Folin-Ciocalteu reagent and 2 ml of 10% Na_2CO_3dissolved in water. Total phenol content was calculated as gallic acid equivalent from a calibration curve. The calibration curve was prepared by preparing gallic acid solutions at concentration10, 25, 50, 100, 200 and 250 µg/ml in methanol (50%). Total phenol content is expressed in terms of gallic acid equivalent as mg/g of dry mass.

Colorimetric aluminum chloride method, as described by Dutta [5], was used for flavonoid content determination. Total content of flavonoid was determined in terms of quercetin calibration curve. 100 mg plant sample was dissolved in 10 ml of methanol. The solution was filtered. From the above filtrate 2 ml was taken into a test tube, mixed with 100 µl of 10% aluminum chloride, 100 µl of 1M potassium acetate and 2.8 ml of distilled water. The mixture was then incubated for 30 minutes at room temperature and the absorbance was recorded at 415 nm. To prepare the calibration curve quercetin solution was prepared with different concentrations 5, 10, 25, 50, 80 and 100 µg/ml in methanol. Total flavonoid content was calculated in terms of quercetin equivalent as mg/g of dry mass.

Total alkaloid content was determined by spectrophotometeric method and studied with the help of colchicine standard curve by using 1,10-Phenanthroline, as described by Dutta [5] and Singh [6] with slight modification. 100 mg of powdered leaf, stem and root were extracted separately in 10 ml of 80% methanol and filtered. The filtrate was then allowed to centrifuge at 5000 rpm for 10 minutes and supernatant was collected for further procedure. 1ml of above supernatant was taken into a test tube, mixed with 1ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1 ml of 0.05 M of 1, 10-Phenanthroline solution. The complete mixture was allowed to incubate in hot water bath at 70 ± 2°C for 30 minutes. The absorbance of the red colored mixture was taken at 510 nm. Total alkaloids content were calculated from colchicines calibration curve and expressed in terms of colchicine equivalent as mg/g of dry weight

RESULTS AND DISCUSSION

In this study the methanol extracts of leaves, stems and roots of *Aristolochia roxburghiana* were subjected to evaluate the presence of major secondary metabolites. Table 1 shows the presence of all studied compounds, *viz.*, Alkaloids, flavonoids, terpenoids, phenols, tannins and saponin in all three extracts. Previously, a number of studies have well explained the capability of phenolic compounds such as phenolic acids, flavonoids, etc., to function as antioxidant by scavenging free radicals. Free radical formation leads to oxidative stress which ultimately causes severe pathological conditions such as inflammation, asthma, neuro-degeneration, arthritis, etc., and cardio-vascular problems including ageing affects [7]. Moreover, phenolic compounds act as metal chelators, antimutagens, anticarcinogens and antimicrobial agents [8]. Tannins function as antimicrobial agent as they inhibit the growth of different microorganisms such as yeast, fungi, and some bacteria [9]. Tannins and terpenoids have been reported to function as analgesic and anti-inflammatory. Further, tannins possess the property of astringency [10]. Saponins have been suggested as anti-carcinogens and its mechanism include direct cyto-toxicity and immune modulator effects. Alkaloids, one of the major groups of secondary metabolites obtained mostly from higher plants, have been used as drug due to its antimicrobial property [10]. Moreover, the pharmacological action of alkaloids varies widely: some (morphine, codine) are analgesics and narcotics while others (strychnine, brucine) are central nervous stimulants. Some (atropine, homatropine) are mydriatics whereas others (physostigmine, pilocarpine) are myotics. Some (ephedrine) cause a rise in blood pressure but others (reserpine) produce a fall in excessive hypertension. In fact, the alkaloids are capable of extensive physiologic activity. Alkaloids are highly reactive substances with biological activity in low doses [11].

Total amount of phenol, flavonoid and contents were calculated from gallic acid ($y = 0.022x - 0.003$, $R^2 = 0.998$), quercetin ($y = 0.023x - 0.023$, $R^2 = 0.997$) and colchicines ($y = 0.000x + 0.308$, $R^2 = 0.990$) standard curves (Figures 3-5). The total phenol contents in their extract were found 16.65, 4.48 and 8.18 mg/g for leaf, stem and root respectively in terms of gallic acid equivalent. Similarly, the total flavonoid contents were found 6.88, 2.19 and 6.31 mg/g for leaf, stem and root respectively in terms of quercetin equivalent. Likewise, the total alkaloid contents were 393.5, 220 and 400 mg/g for leaf, stem and root respectively in terms of colchicine equivalent (Table 2). The presence of phenols, flavonoids and alkaloids contents was well confirmed with qualitative investigations. However, the total phenols (range from 4.48 to 16.65 mg/g) and flavonoids (range from 2.19 to 6.88 mg/g) contents were found to be comparatively low which can be attributed the moderate in antioxidant activity. But, the total alkaloids content (range from 220 to 400 mg/g) is comparatively high which is encouraging and indicates the potential to act as sources of wide range of drugs.

Table 1: Secondary metabolites constituents in the methyl alcoholic extract of different plant parts of *A. roxburghiana* Klotzsch

Secondary metabolites	Chemical tests	Plant parts used	Indication: '+' for presence '-' for absence
		Root	+
Alkaloids	Mayer's test	Stem	+
		Leaf	+
		Root	+
	Dragendroff's test	Stem	+
		Leaf	+
Flavonoids		Root	+
	Alkaline test	Stem	+
		Leaf	+
		Root	+
	Shinoda test	Stem	+
		Leaf	+
Phenols		Root	+
	Ferric chloride test	Stem	+
		Leaf	+
Tannins		Root	+
	Lead acetate test	Stem	+
		Leaf	+
Terpenoids		Root	+
	Salkowski's test	Stem	+
		Leaf	+
Saponins		Root	+
	Foam test	Stem	+
		Leaf	+

Figure 1: *A. roxburghiana* klotzsch

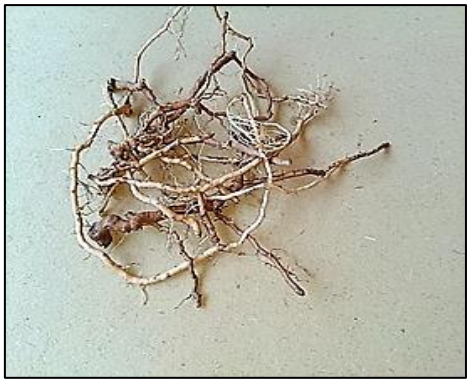

Figure 2: Roots of *A. roxburghiana* Klotzsch

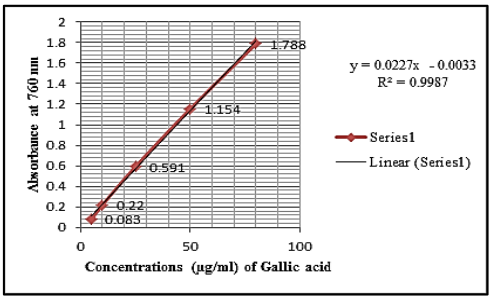

Figure 3: Standard calibration curve of gallic acid for the determination of total phenol content

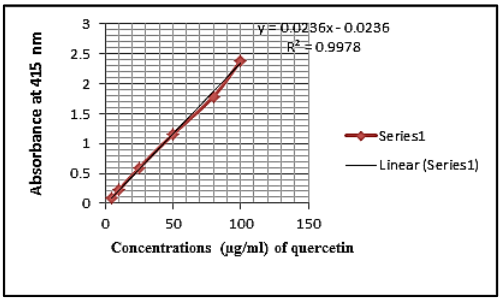

Figure 4: Standard calibration curve of quercetin for the determinationof total flavonoid content

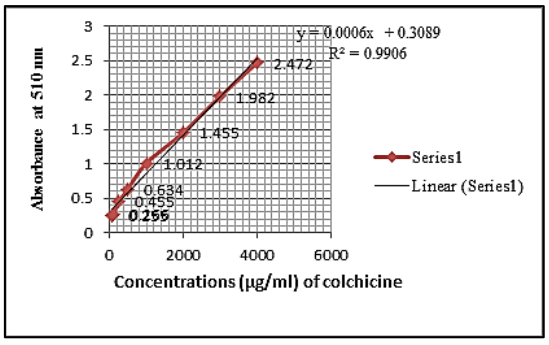

Figure 5: Standard calibration curve of colchicine for the determinationof total alkaloid

Table 2: Total amount of phenol, flavonoid and alkaloid contents of *A. roxburghiana* Klotzsch

Methanol extracts of plant parts	Total phenol (in mg/g, gallic acid equivalent)	Total flavonoid (in mg/g, quercetin equivalent)	Total alkaloid (in mg/g, colchicine equivalent)
Leaf	16.65	6.88	393.5
Stem	4.48	2.19	220
Root	8.18	6.31	400

CONCLUSION

In conclusion, the results suggest that a decrease in viability and an increased activity of caspases 3 and 7 in the colon cancer cell lines SW480 and $Caco_2$ were induced by phytochemicals present in the ethanolic extract of *P. edulis*. For this reason, further research needs to be conducted, to study the chemopreventive properties of *P. edulis* for the treatment of colorectal cancer.

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