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Study of secondary metabolites of *Gomphostemma niveum* Hook.f. in Assam, India

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Abstract

Gomphostemma niveum Hook.f., an ethnomedicinal plant of Assam, India, was screened for secondary metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannins and saponins, and total phenol, flavonoid and alkaloid contents were also studied. It was found that the methanol extract of leaves contains all the screened compounds. The total phenol, flavonoid and alkaloid contents were found 75.04 mg/g, 28.23 mg/g and 0.083 mg/g in terms of gallic acid, quercetin and colchicine equivalent respectively. Findings of the study provided evidence that crude extract of the plant species contains medicinally important bioactive compounds and justifies the uses of the plant in the traditional medicine for the treatment of different diseases.

Keywords: *Gomphostemma niveum*, ethnomedicinal plants, secondary metabolites.

1. Introduction

Secondary metabolites, a group of bioactive substances, having diverse classes of organic compounds like alkaloids, terpenoids, phenols, flavonoids, tannins, saponins, etc., are produced through secondary metabolism in different plants. The medicinal value of plants lies in these chemical substances that have definite physiological action on the human body¹. Phytochemical analysis of ethnomedicinal plants for secondary metabolites is an important area of fundamental research because of its relevance for the discovery of therapeutic agents and providing clues for new sources of bioactive compounds^[2-3].

Assam, a state in the North-Eastern region of India, with a geographical area of 78, 438 sq. km. (88°25' – 96°0' E latitude and 24°5' – 28° 0' N longitudes) with hills and plains, is known for its rich flora and diverse forests and vegetations due to its unique topography, climate and altitude patterns. This region of India is also a homeland of people belonging to more than 100 ethnic tribes and sub-tribes, and accordingly has been endowed with rich traditional knowledge^[4]. *Gomphostemma niveum* Hook.f., locally known as 'Bhumi-tita', belonging to family Lamiaceae, is an herbaceous perennial plant found in Assam (Photo plate 1). The plant has several ethno-medicinal applications. An infusion (100 ml) prepared from the leaves is given orally twice a day before meal for 15 days in tuberculosis. A decoction prepared from the leaves (25 g) in 1000 ml water, is given orally twice a day in malaria fever. Leaf-paste is applied locally in insect stings. However, the plant is less known to the literature of Indian Medicinal Plants^[5-11]. While, in the line of phytochemical studies on this plant species, a few works have been reported from abroad^[12], however, works have been found to be scanty from this region. Realizing the medicinal significance of the plant species, the present study was undertaken for screening of secondary metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannins, saponins and to study the total phenol, flavonoid and alkaloid contents.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh leaves of the plant species were collected from the homestead garden. Taxonomic identification was carried out with the help of regional floras and monographs^[13-15], and also compared with the herbarium present in the North East Institute of Science and Technology, Jorhat, Assam and India. Voucher specimen (Accession No. B.D. 1021) is deposited in the Herbarium of Sibsagar Girls' College, Sivasagar, Assam and India.

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2.2 Preparation of Extract of Plant Material

The leaves were washed with running tap water, leaf material was then air dried under shade and after complete drying the material was grained in mixer. Plant extract was prepared using methanol as extracting solvent. 100 g of the dried and powdered plant material (leaf) was extracted with 400 ml of methanol at 65 °C for 2 days using Soxhlet extraction method. After filtering and evaporating to dryness, the crude methanolic extract was obtained.

2.3 Phytochemical Screening for secondary metabolites

Chemical tests were carried out qualitatively on the extract following standard procedures to identify the phytochemical constituents [16-17].

a. Test for alkaloids

Dragendroff's test: In a test tube containing 1 ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

Mayer's test: To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate indicates the presence of alkaloids.

Wagner's test: To 1 ml of the extract, 2 ml of Wagner's reagent was added. Appearance of a reddish brown precipitate indicates the presence of alkaloids.

Hager's test: Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

b. Test for flavonoids

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Lead acetate test: To the test solution, a few drops of lead acetate solution were added. Formation of yellow precipitate indicated the presence of flavonoids.

c. Test for phenolic compounds

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

d. Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

e. Test for terpenoids

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance

of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour of interface after addition of conc. sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

f. Test for saponins

Foam test: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously then some drops of olive oil were added. The formation of stable foam was taken as an indication for the presence of saponins.

2.4 Determination of total phenol, flavonoid and alkaloid contents

Folin-Ciocalteu method, as described by Nabavi *et al.* [18], was used for phenol content determination. Briefly, 0.5 ml (1 mg/ml) of plant extract was diluted to 5 ml with distilled water. Folin-Ciocalteu reagent 5 ml (1:1 diluted with distilled water) was added and mixed thoroughly. After five minutes 5 ml of 10% Na₂CO₃ solution was added. The solution was warmed for one minute, and then cooled. The absorbance of the reaction mixtures was measured at 760 nm with UV-Visible spectrophotometer. Total phenol content was calculated as gallic acid equivalent from a calibration curve. The standard curve was prepared by 50, 100, 150, 200 and 250 µg/ml solutions of gallic acid in methanol: water (50:50 v/v). Total phenol value is expressed in terms of gallic acid equivalent as mg/g of dry mass.

Colorimetric aluminum chloride method, as described by Nabavi *et al.* [18], was used for flavonoid content determination. Briefly, 0.5 ml (1 mg/ml) of plant extract in methanol was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The solution was vigorously shaken and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with UV-Visible spectrophotometer. Total flavonoid content was calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol. Total flavonoid value is expressed in terms of quercetin equivalent as mg/g of dry mass.

Total alkaloid content was determined by spectrophotometric method and studied with the help of colchicine standard curve by using 1,10-Phenanthroline, as described by Singh *et al.* [19]. 100 mg leaf powder was extracted in 10 ml 80% methanol. This was filtered through muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant obtained was used for estimation of alkaloids. The reaction mixture was prepared by mixing 1 ml plant extract, 1 ml of 0.025 M FeCl₃ in 0.5 M HCl and 1 ml of 0.05 M of 1, 10-Phenanthroline in methanol. The mixture was incubated for 30 minutes in hot water-bath with maintained temperature of 70 ± 2 °C. The absorbance of red colored complex was measured at 510 nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of colchicines (0.1 mg/ml, 10 mg dissolved in 10 ml methanol and diluted to 100 ml with distilled water). Total alkaloid value is expressed in terms of colchicine equivalent as mg/g of dry weight.

The total alkaloid content of the sample was calculated by using following formula:

Total alkaloid contents, $C = c \times V / M$

Where,

c = Concentration of the testing solution from standard curve,

V = Volume of extract used during assay,

M = Mass of the plant sample used for extract preparation.

3. Result and Discussion

The present study carried out on the methyl alcoholic extract of leaves revealed the presence of medicinally active secondary metabolites. The result is summarized Table 1. All the studied compounds i.e., Alkaloids, flavonoids, terpenoids, phenols, tannins and saponins were found to be present.

Various experiments have been demonstrated that phenolic compounds such as flavonoids, phenolic acids, etc. are potential antioxidant and antioxidant activity of these compounds is due to their ability to scavenge free radicals. Accumulation of free radicals can cause pathological conditions such as asthma, arthritis, inflammation, neurodegeneration, heart disease, aging effect, etc. [20]. Additionally, phenolic compounds act as (i) metal chelators, (ii) antimutagens and anticarcinogens, (iii) antimicrobial agents [21]. The growth of many fungi, yeasts and bacteria was inhibited by tannins [22]. Further, tannins and terpenoids are attributed for analgesic and anti-inflammatory activities. Apart from these,

tannins contribute property of astringency i.e., faster the healing of wounds and inflamed mucous membrane [23]. Saponins, present in plants, have been suggested as possible anti-carcinogens. The proposed mechanisms of anti-carcinogenic properties of saponins include direct cytotoxicity, immune modulator effects. Likewise, alkaloids are a diverse group of secondary metabolites found to have antimicrobial activities by inhibiting DNA topoisomerase [24].

Total amount of phenol, flavonoid and contents were calculated from gallic acid ($y = 0.005x - 0.083$, $R^2 = 0.995$), quercetin ($y = 0.004x + 0.040$, $R^2 = 0.994$) and colchicines ($y = 0.000x + 0.055$, $R^2 = 0.986$) standard curves (Figure 1, 2 & 3). The total phenol, flavonoid and alkaloid contents in their extract were found 75.04 mg/g, 28.23 mg/g and 0.083 mg/g in terms of gallic acid, quercetin and colchicine equivalent respectively (Table 2). The phenol, flavonoid and alkaloid contents were well confirmed with qualitative investigations. Although, the total amount of flavonoid content (28.23 mg/g) found to be moderate, but, amount of the total phenol content (75.04 mg/g) is comparatively high which is encouraging and indicates the antioxidant potential of the crude extract. Likewise, total alkaloid content (0.083 mg/g) is also found to be comparatively high indicating the antimicrobial potential of the plant species.

Table 1: Secondary metabolites constituents in the methyl alcoholic extract of leaves of *G. niveum* Hook.f.

Secondary metabolites	Chemical tests	Indication: '+' for presence
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Flavonoids	Alkaline test	+
	Shinoda test	+
Terpenoids	Lieberman test	+
	Salkowski test	+
Phenols	Lead acetate test	+
	Ferric chloride test	+
Tannins	Ferric chloride test	+
	Alkaline test	+
Saponins	Frothing test	+

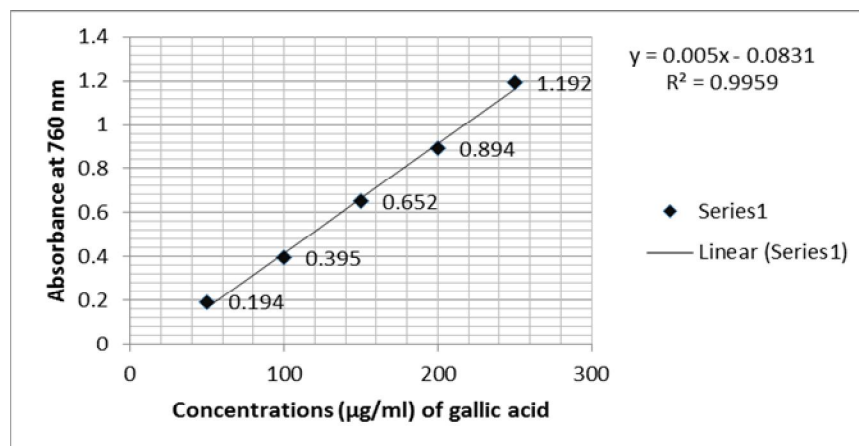


Fig 1: Standard calibration curve of gallic acid for the determination of total phenol content.

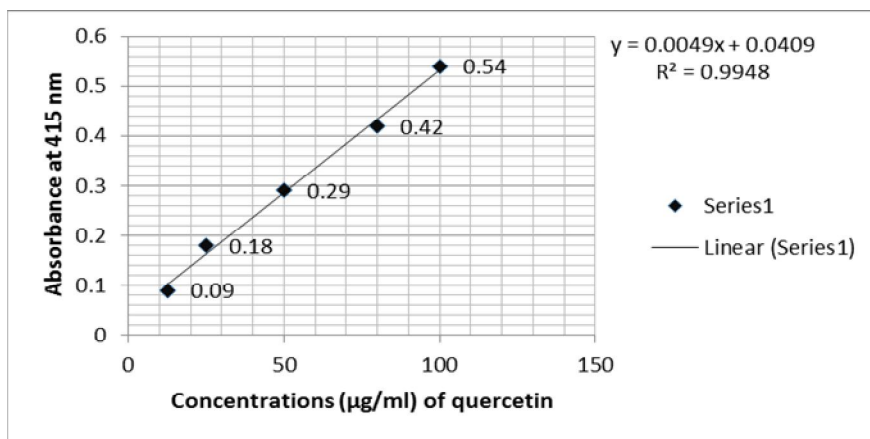


Fig 2: Standard calibration curve of quercetin for the determination of total flavonoid content.

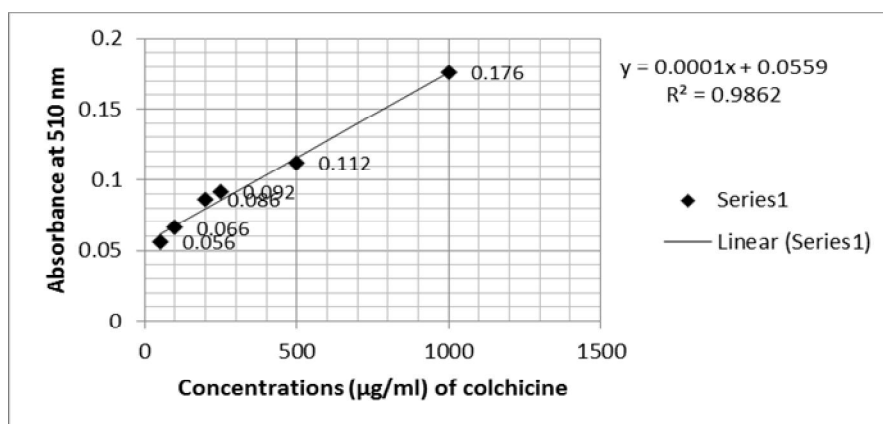


Fig 3: Standard calibration curve of colchicine for the determination of total alkaloid.



Plate 1: *Gomphostemma niveum* Hook.f.

Table 2: Total amount of phenol, flavonoid and alkaloid contents of *G. niveum* Hook.f.

Plant part/ Extract name	Total phenol (in mg/g, gallic acid equivalent)	Total flavonoid (in mg/g, quercetin equivalent)	Total alkaloid (in mg/g, colchicine equivalent)
Leaves/ Methanol extract	75.04	28.23	0.083

4. Conclusion

The plant species screened for secondary metabolites and the total phenol, flavonoid and alkaloid contents seemed to have the potential to act as a source of drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. It is also suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of the plant species.

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