Original Article

Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of *Byttneria herbacea* Roxb

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Abstract

Background: *Byttneria herbacea* Roxb., family Sterculiaceae, commonly called as '*Samarakhai'* in local Odia language, is one of the reputed folklore medicinal herb. Its roots, leaves, and whole plant parts are reported for traditional use in the management of dysentery, sores, cuts, wounds, cholera, leucorrhoea, fractures, and sprains. **Aim:** The aim of the present work was to assess the total tannin content, total alkaloid content (TAC), total phenolic content (TPC), and total flavonoid content (TFC) in root, leaf, and whole plant of *B. herbacea*. **Materials and methods:** The amount of total tannins was analyzed using titrimetric method and total alkaloids by gravimetrical method. TPC was measured using Folin-Ciocalteu's method and calculated as gallic acid equivalents and the amount of total flavonoids by aluminum chloride colorimetric method and calculated as chrysin equivalents. **Results:** Tannin content was found maximum in the leaf (8.148% w/w) followed by whole plant (3.886% w/w) and root (1.553% w/w); similarly, TAC in the leaf (2.306% w/w) was more than those in root (0.814% w/w) and whole plant (1.319% w/w). The TPC of the methanolic extract of root (372.33 ± 14.29 mg/g) was more than whole plant (267.33 ± 7.63 mg/g); The TFC of the methanolic extract of leaf (620 ± 50 mg/g) was found maximum followed by root (553.33 ± 28.86 mg/g) and whole plant (536.66 ± 28.86 mg/g). **Conclusion:** The result of study emphasized presence of tannin, alkaloid, phenol, and flavonoid contents in the root, leaf, and whole plant of *B. herbacea* where the leaf was found to be richest source.

Keywords: *Byttneria*, phenols, *Samarakhadyam, Samarakhai,* spectrophotometric

Introduction

Plants are sources of a variety of bioactive chemical compounds which elicit many pharmacological effects. These bioactive compounds are usually referred to as secondary metabolites which are known to be agents of plant therapies.[1] Bioactive constituents derived from plant extracts have been reported scientifically for various biological activities. The most important biologically active ingredients are phenols, alkaloids, flavonoids, steroids, glycosides, tannins, etc. These chemical compounds are mostly responsible for the desired beneficial properties.[2] The tannin-containing plant extracts are used as astringents, diuretics, against stomach and duodenal tumors,[3] and as antioxidant, antiseptic, anti-inflammatory, and hemostatic pharmaceuticals.[4] Topical applications of tannins help drain out all irritants from the skin. In particular, tannins-rich remedies are used as antioxidants,[5] anthelmintic,[6] antivirals, and antimicrobials.[7] Alkaloids are important for the protecting and survival of plant due to the fact they may sure their survival against microorganisms (antibacterial and antifungal activities), insects, and herbivores by the way of capacity of allopathically active chemicals.[8]

Phenolic acid possesses numerous biological activities such as antioxidant, anti-inflammatory, antimicrobial,

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antihypertensive, anti-carcinogenic, and anti-mutagenic still as ability to change the gene expression,[9,10] etc. Flavonoids are referred to as nutraceuticals on account of a broad spectrum of pharmacological activities in human body. Flavonoids are declared to possess several useful properties, containing antioxidant activity, anti-inflammatory activity, anti-allergic activity, estrogenic activity, antimicrobial activity, vascular activity, and cytotoxic antitumor activity.[11] Plant phenolics and flavonoids are extensively distributed in plant tissues and contribute an important function in free radical scavenging and antioxidant activity. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the chance of diseases.[12]

India has an ancient heritage of traditional medicine. Materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products.[13] Even though such plants are used by traditional physicians, a scientific study of many plants is lacking. *Byttneria herbacea* Roxb., family Sterculiaceae, one among the reputed folklore medicinal herb, is found in peninsular India from Gujarat southwards to Tamil Nadu and in Odisha and Bihar.[14,15] It is locally known as '*Samarakhai'* by tribal people of Odisha and has been used for the therapeutic purposes. Its root, leaf, and whole plant are used to treat different disease conditions like dysentery, sores, cuts, wounds, lactation complaints, syphilis, cholera, leucorrhoea, fracture of limbs, and sprains.[16,17] The major reported activities of the plant are anti-edemogenic,[18] anti-inflammatory activity,[19] anti-asthmatic activity,[20] and anti-oxidant activity.[21] Based on the strong evidence of many pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, wound healing activity of tannin, alkaloid, phenolic, and flavonoid components, the present study was carried out to evaluate the total tannin, alkaloid, phenolic, and flavonoid contents of *B. herbacea* Roxb.

Materials and methods

Chemicals and reagents

Chemicals utilized for study were procured from Rankem Mumbai and Analar India. Gallic acid standard was obtained from Loba chemia, Mumbai, and Folin-Ciocalteu reagent was procured from SRL, India. All chemicals used for analysis were of laboratory grade.

Collection, authentication, and preservation of the samples

Samarakhai, growing in Gandhamardan hill ranges of Paikmal, Bargarh district of Odisha, was identified as *B. herbacea* Roxb., family Sterculiaceae, on the premise of its morphological characters, comparing with the reported characters denoted in Flora of Orissa[15] and with the assist of local taxonomist. The fresh plants were collected within the month of September 2017 from its natural surroundings. Plant specimen is authenticated by Botanical Survey of India (BSI) Kolkata with letter no. CNH/2016/Tech.II/68. Herbarium was prepared and submitted to museum of Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar, vide herbarium no. Phm. 6200/16-17. The collected plant samples were shaken to dispose of adherent soil and dirt. The roots and leaves were separated from the stem. Individual plant part as well as entire plant was washed under running fresh tap water and then with RO water. Then, it had been dried under the shade and powdered with mechanical grinder and sieved through 60#. Powders were stored in air-tight glass container.

Estimation of total tannin content

Total tannin content was estimated by titrimetric method as recommended by the API (Ayurvedic Pharmacopoeia of India).[22]

Estimation of total alkaloid content

Total alkaloid content (TAC) was estimated by gravimetric method.[23]

Extract preparation

Exactly weighed 5 g of root, leaf, and whole plant powder of *B. herbacea* macerated with methanol (100 ml), keeping it for overnight with initial occasional shaking up to 6 hrs, and then set aside. After 24 hours, it was filtered and methanolic extract was collected. Then, the prepared methanol extract was taken for quantitative estimation.

Estimation of total phenolic content

Standard preparation

About 10 mg each of gallic acid was accurately weighed into clean and dry volumetric flasks, dissolved in methanol, and the volume was made up to 10 ml using the same solvent to make the concentration of the solution as 1 mg/ml.

Test sample preparation

A stock solution of the test substance was prepared by dissolving 10 mg of dried methanolic extract in 10 ml methanol to give concentration of 1 mg/ml.

Spectrophotometric method

Estimation of total phenol content in the root, leaf, and whole plant methanolic extract was measured spectrophotometrically by Folin–Ciocalteu colorimetric method,[24,25] using gallic acid as the standard and expressing results as gallic acid equivalent (GAE) per gram of sample.

Estimation of total flavonoid content

Standard preparation

About 10 mg each of chrysin was accurately weighed into clean and dry volumetric flasks, dissolved in methanol, and the volume was made up to 10 ml using the same solvent to make the concentration of the solution as 1 mg/ml.

Test sample preparation

A stock solution of the test substance was prepared by dissolving 10 mg of dried methanolic extract in 10 ml methanol to give concentration of 1 mg/ml.

Spectrophotometric method

The total flavonoid content (TFC) of the root, leaf, and whole plant methanolic extract was determined by aluminum chloride colorimetric assay.[26]

Results and Discussion

Medicinal plants since ancient times are lauded for their numerous pharmacological actions that might be attributed to the presence of secondary plant metabolites like alkaloids, phenols, flavonoids, tannins, and steroids. They have played a significant role worldwide in preventing and curing varied human ailments due to their vast spectrum of chemical and biological activities.

The results of the quantitative evaluation of tannins and alkaloids are presented in Table 1. Maximum tannin was found in the leaf (8.148% w/w) followed by the whole plant (3.886% w/w) and root (1.553% w/w). TAC was found maximum in the leaf (2.306% w/w) followed by the whole plant (1.319% w/w) and root (0.814% w/w). This concurs with the familiar information that secondary metabolites are normally concentrated in the leaf.

The phytochemical screening of the chemical constituents of extract of *B. herbacea* reveals that the plant usually includes the major secondary metabolites in moderate abundance. These phytochemicals were recognized to have medicinal physiological effects.[27] Previously, it was noted that the potential of alkaloids as effective medications and linked it to their sedative qualities and significant nervous system effects.[28] The reasonable amount of alkaloids in the various parts of *B. herbacea,* therefore, seems to support the effectiveness of the plant's usage in ethnomedicinal practice.

Tannin content ranged from 1.553% to 8.148% in samples of *B. herbacea*, which looks incredibly low if the plant is to be used as a source of tannins by businesses like the pharmaceutical industry. However, consuming too many tannins could be harmful to humans. This is due to the fact that tannins are chelators for metal ions, and because tannin-chelated metal ions are not bioavailable, they may reduce iron's bioavailability and cause anemia. El-Waziry *et al*. had previously linked regular ingestion of specific plants with high tannin concentrations to esophageal cancer in humans.[29] As a result, the tannin concentration in *B. herbacea* might not be sufficient to cause excessive toxicity, making it suitable for use as nutraceutical product. Numerous tannin components have been implicated as being anti-carcinogenic and antimutagenic. The anti-oxidative characteristic of tannins, which is critical in preventing cellular oxidative damage, including lipid peroxidation, may be related to their anticarcinogenic and antimutagenic potential. Tannin's antibacterial qualities can potentially be employed in food processing to extend the shelf life of some foods. Tannins may also have additional physiological effects, including those that speed up blood clotting, lower blood pressure, lower serum cholesterol levels, and modify immune responses.[30]

The results of the total phenolic content and TFC are presented in Table 2. The content of the phenolic compounds in methanolic extract of root, leaf, and whole plant, determined from regression equation of calibration curve ($y = 0.0209 \times -0.0472$, $R^2 = 0.9996$), and are being expressed in GAE were 372 ± 14.29, 0, 267.33 ± 7.63, respectively. The content of flavonoids (mg/g) in methanolic extract of leaf, root, and whole plant, in chrysin equivalent determined from regression equation of calibration curve ($y = 0.002x - 0.0044$, $R^2 = 0.9911$) were 620 ± 50, 553.33 ± 28.86, 536.66 ± 28.86, respectively. The standard calibration curves of gallic acid and chrysin are shown in Figures 1 and 2, respectively.

Phenolic compounds are well-known for being potent antioxidants that can break chains. Because of their ability to scavenge free radicals due to their hydroxyl groups, phenols are essential components of plants. The antioxidant effect of the phenolic compounds may directly depend on them. When consumed up to 1g daily from a diet high in vegetables and

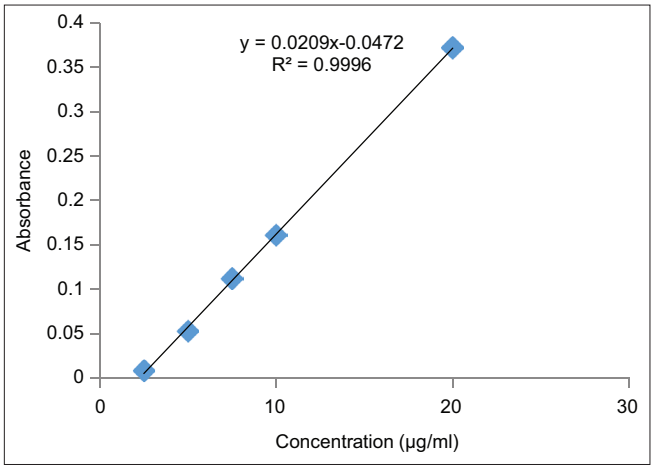

Figure 1: Standard calibration curve for total phenolic content for standard gallic acid

Table 1: Total tannin and total alkaloid contents of *Byttneria herbacea*

Part used	Total tannin content (% w/w)	Total alkaloid content (% w/w)
Leaf	8.148	2.306
Root	1.553	0.814
Whole plant	3.886	1.319

Table 2: Total phenol and flavonoid contents of *Byttneria herbacea*

Part used	Total phenolic content, mg/g plant extract (GAE)	Total flavonoid content, mg/g, plant extract (CE)
Leaf	0	620±50
Root	372.33±14.29	553.33±28.86
Whole plant	267.33±7.63	536.66±28.86

Values are presented as mean±SD (n=3). SD: Standard deviation, GAE: Gallic acid equivalent, CE: Chrysin equivalent

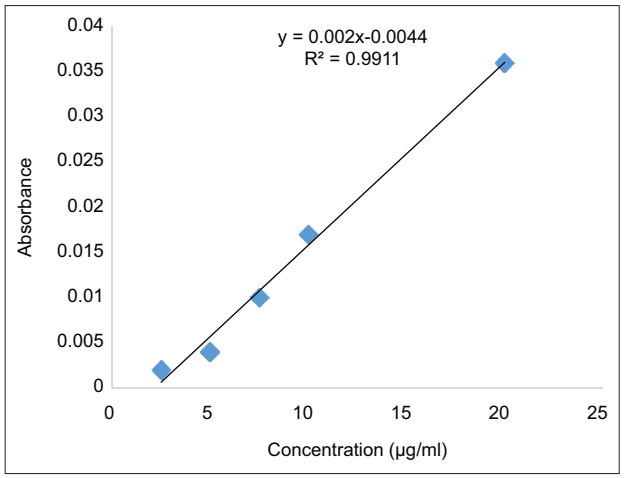

Figure 2: Standard calibration curve for total flavonoid content for standard chrysin

fruits, polyphenolic substances may have inhibitory effects on mutagenesis and carcinogenesis in humans.[31] Total phenols, which are important in regulating oxidation, were found to be present in detectable amounts in the root and whole plant samples of *B. herbacea*. The *B. herbacea* root can be employed as a convenient source of natural antioxidants, according to the study's findings.

Flavonoids are a group of secondary metabolites that persist in plants in the form of polyphenolic molecules or glycoside linkages, which are both polar soluble and aqueously soluble chemical compounds. All *B. herbacea* samples were discovered to have an adequate level of total flavonoids. Since flavonoids have antioxidant properties, they may provide protection from cancer and heart disease[32] likely by boosting the body's defense against pathology induced free radicals production.[33] Thus, they offer compelling data to support traditional claims about *B. herbacea*.

The results showed that the test drug sample contains the potent amounts of tannin, alkaloid, phenolic, and flavonoid compounds. Many of the therapeutics properties of this plant may depend on these phytoconstituents. These findings supported the traditional uses of *B. herbacea* Roxb. for treating different disease conditions like dysentery, sores, wounds, syphilis, cholera, leucorrhoea, fracture of limbs, and sprains. Phytochemical studies to isolate components are also needed to be done to find the molecules liable for pharmacological activity. In addition, further spectral characterization of the isolated compounds will yield promising drug of future use.

Conclusion

The present study has shown that *B. herbacea* leaf, root, and whole plant are rich in tannin, alkaloid, phenolic, and flavonoid compounds, and therefore provided some biochemical basis for its ethnomedicinal uses. Moreover, it can also be concluded that *B. herbacea* leaf having highest concentration of tannins, alkaloids, and flavonoids compared to root and whole plant. As a promising source of bioactive compounds, it can be an excellent source of valuable and nutritional drugs and liable for the treatment of varied diseases. Further studies are necessary to isolate and characterize the bioactive principles.

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Conflicts of interest

There are no conflicts of interest.

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