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Preliminary Phytochemical Screening from Different Parts of *Bauhinia tomentosa* L. And *Bauhinia malabarica* Roxb. (Caesalpiniaceae)

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Article

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ABSTRACT

In Indian traditional system of medicine, importance of *Bauhinia* species is well evidenced from earlier literature. Tender leaves, flowers and young pods of *B. tomentosa* and *B. malabarica* are consumed by various ethnic groups in India. In the present study, edible and therapeutically important plant parts of both the species were extracted with different solvents systems. Acetone extract of both the plants yielded substantial amounts of total phenolics, tannins, condensed tannins, flavonoids and vitamin C. The flower extracts of *B. tomentosa* registered higher phenolic, tannin and vitamin C contents, thereby justifying their traditional usage in medicine. Similarly, the seed extracts of these two species also exerted significantly ($P<0.05$) higher flavonoid and condensed tannin contents. Therefore on the basis of the present findings, both of the species of *Bauhinia* can be considered as a promising source in health, food and pharmaceutical industry.

INTRODUCTION

From the beginning, combating disease has been an important aspect of interactions between human beings and the natural environment, and plants have forever been a catalyst for our healing. It contains a bewildering diversity of secondary metabolites often with very attractive bioactivities. They are described as chemical factories that are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances whose structures could escape the imagination of synthetic chemists. Therefore search for novel phytochemicals in plants is the most valuable source of new bioactive chemical entities to benefit mankind. Among the large number of medicinal plants, the genus, *Bauhinia* (*Caesalpiniaceae)* have been studied extensively in recent years with a surging interest with reference to its medicinal values. This genus comprises of more than 300 species and is mainly found in the tropical areas. Phytochemical and pharmacological studies carried out with Bauhinia species for various parts have demonstrated the presence of several classes of organic compounds of medicinal interest. They are used in various indigenous systems of medicine and are popular among the various ethnic groups for the cure of various ailments.

The species *Bauhinia tomentosa* L. is a small scrambling shrub that grows throughout southern India, Assam and Bihar. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. In Ayurveda, the plant parts are recommended for the treatment of snake bite and scorpion-sting. Seeds when made into a paste with vinegar have an efficacious application to wounds inflicted by poisonous animal's viz., snakes and scorpions [1]. The dried leaves, flower buds and a decoction of the root and bark were used medicinally by the doctors of south Africa[1,2] . Leaves, buds and flowers are edible. In folklore medicine, they are used to treat ailments such as headache, malaria, dysentery and diarrhoeal affections. The bruised bark is applied externally for tumors

and wounds such as scrofulous. In India, decoction of the root bark is used as a vermifuge and an infusion of the stem bark as an astringent gargle. In India and Sri Lanka, the root bark is administered internally for conditions of the large intestine, and inflammation of the liver. The fruit is diuretic, while seeds are edible used as tonic with aphrodisiac action[1,3].

Similarly, *Bauhinia malabarica* Roxb. is a small deciduous tree, distributed throughout India, mainly in the sub- Himalayan tracts, Bengal, Assam and in south India; areas receiving 1000 to 3000 mm annual rainfall. The leaves of the plant are consumed in India, Indonesia and Thailand, among others. It is used in traditional medicine for wound healing, dysentery, headache, fever and as an emmenagogue[4]. Leaves are acrid, used as a flavoring agent for meat and fish[5,6,7]. The mineral content of the leaves shows that they are a prominent source of calcium and iron. Young shoots are also edible and are used to treat worm infestations, leprosy, wounds, menorrhagia, gout, scrofula, wasting diseases, cough, haemorrhage, urinary disorders, glandular swellings and goiter[2,5]. Despite this interesting health virtue and being a well known natural source of food, meager information is available regarding the phytochemical investigations of these plants. Therefore, the present study was carried out to elucidate the total phenolics, tannin, total flavonoid, condensed tannin and vitamin C content in various parts of *B. tomentosa* and *B. malabarica* in order to explain the multifaceted role of these medicinal plants.

MATERIALS AND METHODS

Plant Material

Fresh leaves, stem, root, flowers, pods and seeds of *B. tomentosa* were harvested from the surrounding areas of Coimbatore city, Tamil Nadu, India. Leaves, stem, pods and seeds of *B. malabarica* Roxb. were collected from Siruvani hills, Coimbatore district, Tamil Nadu, India. The authenticity of the selected plant species were confirmed at Botanical Survey of India, Southern circle, Coimbatore. The voucher specimens (*B. tomentosa* L., vide No. BSI/SC/5/23/08-09/Tech.-1719; *B. malabarica* Roxb., vide No. BSI/SC/5/23/08 -09/Tech.-1718) were lodged in the Department herbarium for future reference. The plant materials from the respective species were cleaned, washed with copious amount of distilled water, shade dried, chopped into bits, and coarsely powdered in a Willy Mill to 60 mesh size (Nippon Electricals, Chennai, India) for extraction.

Preparation of Crude Plant Extracts

50 g of coarsely powdered plant samples were exhaustively extracted with acetone/water (70/30, v/v), followed by methanol/water (50/50, v/v) using a round bottom flask with an attached reflux condenser for 3 h at a controlled temperature. The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE300; Yamato, Japan), lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) to remove traces of water molecules and the lyophilized powders were stored at -20°C until used directly for phytochemical analysis.

Total Phenolics and Tannins

The total phenolic content of plant extracts was determined using Folin-Ciocalteu reagent according to the procedure described by Siddhuraju and Becker[8]. In this method, 20 µg of the extract (dissolved in the respective solvent) was taken in a test tube and made up to the volume of 1.0 ml with distilled water. Then 0.5 ml of freshly prepared Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of 20% sodium carbonate solution were added sequentially in each tube. The mixtures were agitated and left in the dark at laboratory temperature for 40 min for the development of colour. The absorbance was recorded at 725 nm against the reagent blank using a Shimadzu - UV- 160 spectrophotometer (Japan). A calibration curve of gallic acid was constructed, and linearity was obtained in the range of 10-50 µg/ ml. Using the standard curve, the total phenol content of the extract was calculated and expressed as gallic acid equivalent (GAE) mg/ g extract. Using the same extract, tannin content was estimated after treatment with polyvinyl polypyrrolidone (PVPP) as described by Siddhuraju and Manian[9]. One hundred milligrams of PVPP was weighed in a 100 ×12 mm test tube and to this, 1.0 ml distilled water and 1.0 ml of tannin containing phenolic extract was added. The contents were vortexed and kept at 4°C for 15 min. Then the sample was centrifuged (5000 rpm for 10 min at laboratory temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured, as monitored above and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannin content of the extract was calculated as follows:

Tannin (mg GAE/ g extract) = Total phenolics (mg GAE/ g extract) - Free phenolics

(mg GAE/ g extract)

Condensed Tannins

Condensed tannins in the extracts were estimated as described by Porter *et al*[10]. 200 mg of the plant sample was taken in a test tube and to this 10 ml of 70% acetone was added. The contents were placed over night in a shaker. Then they were centrifuged at 5000 rpm for 5 min and the supernatant was collected. 0.5 ml of the supernatant was pipetted out into a test tube, 3.0 ml of the butanol-HCl reagent (95:5 v/v) and 0.1 ml of ferric reagent (2% ferric ammonium sulfate in 2N HCl) were added sequentially. The contents were vortexed and the mouth of each test tube was covered with a glass marble, and then kept in a heating block adjusted at 97 to 100° C for 60 min. After cooling the test tubes, the absorbance was recorded at 550 nm. Suitable blank was subtracted, which is usually the absorbance of unheated mixture. Condensed tannins (% in dry matter) as leucocyanidin equivalent (LE) was calculated by the formula:
Condensed tannins = (Absorbance at 550 nm × 78.26 × Dilution factor) / (% dry matter).

Total flavonoid content

The total flavonoid content was determined spectrophotometrically using the method adopted by Zhishen *et al* [11]. 0.5 ml of appropriately diluted extract solution was mixed with 2.0 ml of distilled water and subsequently with 0.15 ml of 5% sodium nitrite solution and maintained for 6 min. Then, 0.15 ml of 10% aluminium chloride solution was added and allowed to stand for 6 min, and finally 2.0 ml of 4% sodium hydroxide solution was added. Final volume of the contents was made up to 5.0 ml with distilled water and was mixed thoroughly. After 15 min of incubation at laboratory temperature, the absorbance was determined against blank at 510 nm. The total flavonoid content was determined using a standard curve with rutin. The mean of the three values were expressed as milligrams of rutin equivalents (mg RE)/ g extract on a dry weight basis.

Vitamin C (Ascorbic Acid)

For ascorbic acid determination, 10 mg of the dried plant extracts were re-extracted with 10 ml of 1% metaphosphoric acid. They were allowed to stand for 45 min at laboratory temperature and filtered through Whatman No. 4 filter paper. 1.0 ml of the filterate was mixed with 9.0 ml of 50 µM 2, 6-dichloroindophenol sodium salt hydrate and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (Sigma chemicals) and the results were expressed as mg of ascorbic acid equivalent (AA) / 100 g of extract [12].

Statistical analysis

Results were recorded as mean ± standard deviation (SD) (three replicate experiments, n = 3) and subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's Multiple Range Test ($P<0.05$) using statistica (Statsoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants for protection against predators [13]. Plants accumulate phenolic compounds in their metabolism under various stress conditions [14]. It also presents an array of solubility in solvents with different polarity[15]. In the present investigation the calculated yield percentage for different plant parts of *B.tomentosa* and *B. malabarica* varied widely between 1.7 and 27.9% (Table 1). Among the plant parts analyzed, acetone extracts of *B. tomentosa* flower displayed the highest amount of solids recovered (27.9 %) which were in good agreement with the reports of Liu *et al*[16]. It is explained that the high polar solvents are suitable for extracting phenolic constituents effectively [9,17,18].

Phenolic compounds are ubiquitous in plants which exist in several thousand different chemical structures characterized by hydroxylated aromatic ring(s) [19]. They are wide spread virtually in most of the plants, often at high levels. The key role of phenolic compounds as scavengers of free radical is emphasized in several reports[9,20,21]. Using Folin-ciocalteu's reagent, an approximate amount of total phenolic constituents were estimated in the present study. Their values were expressed as milligrams of Gallic Acid Equivalents (GAE) per g of dried samples, using the standard curve of Gallic acid ($R^2 = 0.9913$). The amount of total phenolic and tannin contents varied widely between samples and ranged between 5 and 108 mg GAE /g, and 2 and 77 mg GAE /g sample, respectively. The observed variation might be due to the marked difference in the qualitative and quantitative composition of phenolic compounds and their conjugates present in these extracts[16,22]. Similarly, also demonstrated varying phenolic contents in the extracts of *Xylaria* sp. obtained using different solvents.

Table 1: Total phenolics, tannins, total flavonoids and vitamin C contents of *B. tomentosa* and *B. malabarica* extracts

Plant part	Solvent*	Percentage yield (w/w)		Total Phenolics (mg GAE/g extract)		Tannins (mg GAE/g extract)		Total Flavonoids (mg RE/g extract)		Vitamin C (mg AA /100g extract)	
		B.tomentosa	*B.malabarica*	*B.tomentosa*	*B.malabarica*	*B.tomentosa*	*B.malabarica*	*B.tomentosa*	*B.malabarica*	*B.tomentosa*	*B.malabarica*
Leaf	Ac	19.2	7	49 ± 0.1^{b}	20 ± 0.1^{d}	24 ± 0.2^{b}	12 ± 0.1^{bc}	20.5 ± 2^{d}	18.4 ± 0.3^{d}	221.1 ± 0.5^{c}	98.1 ± 0.5^{b}
	Me	4.2	4.4	10 ± 0.1^{e}	6 ± 0.4^{e}	7 ± 0.1^{de}	4 ± 0.4^{de}	8.3 ± 1^{g}	5.8 ± 0.4^{e}	58.2 ± 0.4^{g}	53.9 ± 0.3^{e}
Stem bark	Ac	3.5	15.6	5 ± 0.4^{g}	95 ± 0.5^{a}	3 ± 0.3^{e}	77 ± 0.3^{a}	10.5 ± 2.7^{f}	64.5 ± 5.1^{b}	28.4 ± 0.3^{j}	179.3 ± 1.1^{a}
	Me	5.5	3.5	8 ± 0.3^{f}	6 ± 0.1^{e}	5 ± 0.1^{e}	4 ± 0.1^{de}	6.3 ± 0.1^{h}	19.5 ± 3.3^{d}	48.1 ± 0.5^{h}	47.7 ± 3.6^{e}
Root	Ac	3	–	5 ± 0.1^{f}	–	3 ± 0.1^{e}	–	10.6 ± 1.7^{f}	–	22.4 ± 0.1^{k}	–
	Me	6.3	–	8 ± 0.1^{f}	–	5 ± 0.1^{e}	–	5.5 ± 0.4^{h}	–	48.6 ± 0.7^{h}	–
Flower	Ac	27.9	–	108 ± 1.3^{a}	–	61 ± 1.3^{a}	–	30.5 ± 2.3^{b}	–	317 ± 2.2^{a}	–
	Me	18	–	50 ± 0.2^{b}	–	27 ± 0.6^{b}	–	15.8 ± 1.2^{e}	–	243.6 ± 1.3^{b}	–
Pod	Ac	5	4.7	20 ± 0.1^{d}	33 ± 0.1^{c}	16 ± 0.1^{c}	16 ± 0.2^{b}	27 ± 0.4^{c}	58 ± 0.7^{c}	61.7 ± 0.1^{f}	63.7 ± 0.9^{d}
	Me	2.8	1.7	8 ± 0.2^{f}	6 ± 0.1^{e}	6 ± 0.2^{de}	2 ± 0.1^{e}	19 ± 1.3^{d}	22 ± 1.3^{d}	38.1 ± 0.5^{i}	24.5 ± 0.1^{f}
Seed	Ac	6.2	5.6	39 ± 0.5^{c}	56 ± 0.4^{b}	30 ± 0.5^{b}	9 ± 0.3^{cd}	43.4 ± 2.6^{a}	70 ± 2.6^{a}	71 ± 0.2^{e}	28 ± 1.5^{f}
	Me	9.4	6.1	18 ± 0.3^{d}	23 ± 0.3^{d}	12 ± 0.3^{cd}	6 ± 0.2^{de}	7.5 ± 1^{g}	21.2 ± 1.1^{d}	124.9 ± 1.4^{d}	74.6 ± 0.6^{c}

*Ac– 70% Acetone; Me – 50% Methanol; '–' = not tested.

Values are mean ± standard deviation (SD) of three independent experiments. Values not sharing a common letter in a column are significantly different ($P < 0.05$).

Table 2: Condensed tannin content in different parts of *B. tomentosa* and *B. malabarica* extracted in 70% acetone.

Sample	*B. tomentosa*	*B. malabarica*
Leaf	14 ± 0.1^{b}	16 ± 0.4^{b}
Stem bark	1 ± 0.3^{d}	21 ± 0.1^{ab}
Root	3 ± 0.4^{d}	–
Flower	23 ± 0.1^{a}	–
Pod	10 ± 0.1^{c}	15 ± 0.2^{b}
Seed	21 ± 0.1^{a}	23 ± 0.2^{a}

'–' = not tested.

Values are mean ± standard deviation (SD) of three independent experiments. Values expressed as mg LE / g dry sample.
Values not sharing a common letter in a column are significantly different ($P < 0.05$).

Among the solvent types examined, 70% acetone extracted almost more of these components with different efficiencies. In particular, both *B. tomentosa* flower (108 mg GAE/g sample) and *B. malabarica* stem bark extracts (95 mg GAE/g sample) contained the highest amount of polyphenolic contents and this elaborates the fact that the polarity of the solvent used for extraction might have profound influence on the solubility of phenolic compounds present in them reported the presence of high molecular weight compounds and flavonoids obtained from 70% acetone fractions[16,23,24]. Siddhuraju and Manian[9] also found that 70% acetone was found to be more efficient solvent for extracting tannins and other phenolic constituents. Therefore it can be concluded that the substantial amounts of these active compounds extracted from the aqueous acetone extracts of the two studied *Bauhinia* species might offer a good source of nutritional antioxidant defence against reactive oxygen species involved in the initiation of deleterious free radical reactions [25,26].

Proanthocyanidins (condensed tannins), are structurally more complex and wide spread phenolic compounds that contribute astringency and bitterness to plants[27]. They are mainly the oligomers and polymers of flavan-3-ols (catechins)[28]. Some authors considered that the polymerized products of flavan-3, 4-diols are also belonging to the category of condensed tannins, called as leucoanthocyanidins[29]. In the present investigation, content of condensed tannins were determined to be present in the range of 1 (*B. tomentosa* stem bark extract) - 23 mg LE /g (*B. tomentosa* flower and *B. malabarica* seeds) (Table 2).

Flavonoids, the most widespread group of natural compounds, occur naturally in a wide range of plant species. They are phenolic derivatives present in substantial amount in plants. In the present study, total flavonoid content of *B.tomentosa* and *B. malabarica* plant part extracts were measured and the values were expressed as mg Rutin Equivalent (RE) /g of the dried sample, generated from the standard curve rutin (R^2= 0.9917). The total flavonoid contents of *B.tomentosa* and *B. malabarica* varied considerably from 5.5 to 70 mg RE /g of dried samples (Table 1). Their concentration varied even in different organs of the same plant which is in agreement with the observations of Justesen and Knethsen ,and Dinelli *et al* [30,31]. The acetone extract of *B. malabarica* seed recorded the highest value of 70 mg RE /g of dried sample followed by its stem bark extracts (64.5 mg RE /g of dried sample) and this fact correlates with the polarity of the solvents used for extraction and solubility of phenolic compounds present in them[9,32]. Moreover, it is presumed that the correspondingly higher amount of flavonoids in polar solvents might be due to the relative composition of water-soluble compounds such as flavonols, viz., rutin, quercetin and kaempferol [33,34,35] already reported in these species [1,4,36]. Food derived flavonoids, especially flavonols (kaempferol and quercetin) are widely occurring flavonoids, known to possess multiple biological functions such as anti-inflammatory, antioxidant, antithrombotic, antiallergic, antiartherogenic, cardioprotective and vasodilatory effects [37].

Like any other dietary compounds, vitamin C (ascorbic acid) is also an important non-enzymatic, water-soluble, chain breaking antioxidant and its contents in different plant parts of *B. tomentosa* and *B. malabarica* were ranging between 22.4 and 317 mg AA/ 100 g extract (Table 1). As vitamin C is water soluble, the polar solvent extracts extracted higher content of it. Noticeably of the different plant parts examined, the acetone extracts of *B. tomentosa* flower exhibited the most appreciable levels of ascorbic acid content (317 mg AA/ 100g sample), followed by their methanolic fractions (243.6 mg AA/ 100g sample); whereas acetone extracts of *B. tomentosa* root contained the minimal levels of vitamin C being detected under the experimental condition used. Based on the active profile exposed through various quantitative assays, it can be concluded that both *B. tomentosa* and *B. malabarica* have favorable amounts of phenolics, tannins, condensed tannins, flavonoids and vitamin C and it is believed that these potential sources of natural antioxidants might offer better protection against oxidative stress generated by free radicals which could in turn maximize their effects on decomposing peroxides, neutralizing free radicals and quenching singlet oxygen species[38,39]. Therefore, the assessment of its antioxidant properties might be a fruitful approach for advocating it in nutraceuticals and therapy.

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