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DETERMINATION OF TOTAL PHENOLIC CONTENT OF THE STEM BARK OF *BAUHINIA VARIEGATA* LINN.; AN APPROACH TO STANDARDIZATION

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ABSTRACT

Bauhinia variegata Linn., commonly known as 'Camel's foot tree or Orchid tree', is widely distributed in most tropical countries and have been used frequently in folk medicine for varied purposes; to treat different kinds of pathologies, particularly diabetes, infections, as well as pain and inflammation owing to the presence of numerous phytochemical constituents residing in the plant. The powerful biological activities as exhibited by plant phenolics and flavonoids posed the need of determining their contents in *Bauhinia variegata* stem bark. The present study was aimed at estimation of total phenolics, flavonoids and tannins in the petroleum ether, ethyl acetate, methanol and aqueous extracts of the stem bark of *Bauhinia variegata*. The contents were determined by spectrophotometric assays by measuring the absorbance at different wavelengths. Total phenolic content were estimated by the Folin–Ciocalteu colorimetric method; the total tannin content was estimated by Folin-Denis method whereas the total flavonoid content was estimated by aluminium chloride colourimetric method. The methanol extract showed highest concentration of phenolics, flavonoids and tannins with petroleum ether extract reporting the least; ranging between 15-89 µg /mg of gallic acid equivalent, 8-135 µg/mg of (±)-catechin equivalents and 15-102 µg/mg of tannic acid equivalents respectively. The results clearly indicate that *B. variegata* is a rich source of phenolics, the basis of its traditional use in different systems of medicines.

Key Words: Total phenolics, flavonoids, antioxidant, stem bark extract

INTRODUCTION

Plants synthesize compounds with biological activity, namely antioxidant, as secondary products, which are mainly phenolic compounds serving in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Phenolics are secondary plant

metabolites ranging from simple to highly polymerized compounds *[1]*. Many epidemiological studies have shown that the consumption of phenolics-rich foods is associated with the prevention of chronic diseases *[2]*. In addition to their antioxidant properties, these compounds have been reported to be potential candidates in lowering cardiovascular diseases *[3]* and anti-carcinogenic activities *[4, 5]* antiallergenic, anti-arthrogenic, anti-inflammatory, antimicrobial and antithrombotic effects *[6]*. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks *[7]*. In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox potential of their phenolic hydroxyl groups that allow them to act as reducing agents, hydrogen-donating antioxidants and oxygen quenchers *[8]*.

The aim of the present study was to evaluate the total phenolic content of the petroleum ether; ethyl acetate; methanol and aqueous extracts of *B. variegata* (BV) stem bark.

The genus *Bauhinia* (Fabaceae, Leguminosae) consists of approximately 300 species, which are commonly known as 'cow's paw' or 'cow's hoof'. They are widely distributed in most tropical countries, including Africa, Asia and South America. Their leaves and stem-bark have been used frequently in folk medicine as a remedy for different kinds of pathologies, particularly diabetes, infections, pain and inflammatory processes. The biological properties of different *Bauhinia* spp. phytopreparations and pure metabolites have been investigated in numerous experimental *in vivo* and *in vitro* models. The secondary metabolites produced by this genus, particularly the flavonoids, make the plants under this genus an important source of potential phytotherapeutic and medicinal agents.

Antioxidant properties elicited by plant species have a full range of perspective applications in human healthcare. In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or teas rich in natural antioxidants *[11]*. Rakta Kanchan (*Bauhinia variegata linn.)* a medium sized, deciduous tree, found throughout India, ascending to an attitude of 1,300 m in the Himalayas imitates a good example. It is traditionally used for treating bronchitis, leprosy, tumours, and ulcers. Its extracts have been found to have antibacterial and antifungal activity *[12]*. Studies carried out with *Bauhinia variegata* have also demonstrated hypoglycaemic activity in laboratory animals *[13]*. An infusion from its bark is used as an astringent and tonic and is useful for treating scrofula, skin diseases, and

ulcers. The decoction of the roots is used in dyspepsia and as an antidote to snake poison *[14]*. All these clinical applications of *Bauhinia variegata* reside in their phytochemical content, mainly, the phenolics and their ability to scavenge free radicals.

Since these phenolic compounds have now become the key ingredient for most herbal formulations, quantising their amounts is also necessary. The present evaluation of various quantitative standards will be helpful for standardizing the drug for its various pharmacological potentials, to ascertain its identity, to establish the quality and purity of this plant material in closely related species and to check the adulteration.

MATERIAL AND METHOD:

Procurement of plant material:

The stem bark collected from the local area of Garhwal was authenticated by Botanical Survey of India, Dehradun. A voucher specimen was deposited at the Department of Pharmacy, Guru Ram Das Institute of Management and Technology, Dehradun for future reference.

Preparation of extract:

Stem bark were separated from wood with the help of an axe and used for extraction. The collected materials were washed thoroughly in water, chopped, air-dried for a week at 35-40°C and pulverized in electric grinder. The powdered plant material was subjected to successive extraction with petroleum ether, ethyl acetate, methanol and water using soxhlet extractor for 24 hours with each solvent. The extracts were concentrated under reduced pressure in a rotary evaporator and dried. The percentage extractive values were recorded.

Determination of Total Phenolic Content *[15]*:

The total phenolic content of the extracts was evaluated by spectrophotometric method measuring the absorbance at 765 nm. One milliliter of sample (concentration 1 mg/mL) was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and made up to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (20–100 lg/mL, $Y = 0.025x + 0.0378$, $R^2 = 0.997$) and the results were expressed as µg of gallic acid equivalents/ mg of extract (GAE).

Determination of Total Flavonoid content *[15]*:

Flavonoid content in the extracts was determined by spectrophotometric method. The extract (concentration 1 mg/mL) was

mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of 10% $AlCl_3$ solution was added. After 6 min, 500 µL of 1M NaOH and 275 µL of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (±)-catechin was used for constructing the standard curve (20–180 lg/mL, Y = 0.0012x + 0.0541, R^2 = 0.999) and the results were expressed as µg of (±)-catechin equivalents (CE) per mg of extract.

Determination of Total Tannin Content *[16]***:**

Tannin content of the extracts was measured by Folin–Denis method. The various extracts (50 lL) were made up to 7.5 mL by adding double distilled water. 0.5 mL Folin–Denis reagent and 1 mL of Na_2CO_3 were added to it. Again the volume was made up to 10 mL with double distilled water. Absorption was recorded at 700 nm. Tannic acid was used to calculate the standard curve (20–100 µg/ mL, Y = 0.065x + 0.0011, R^2 = 0.998) and the results were expressed as µg of tannic acid equivalents (TAE) per mg of extract.

RESULT:

BV stem bark showed highest extractive value with methanol followed by water. Least extractive value was reported with petroleum ether. (Table 1)

Table 1. Extractive values of *Bauhinia variegata* stem bark

Extract	Extractive Value (% w/w)
Petroleum ether Extract	1.63
Ethyl Acetate Extract	3.44
Methanol Extract	9.2
Aqueous Extract	6.89

Total phenolic content

The methanol extract recorded the highest content of about 89 µg/mg of extract. The next highest was observed in aqueous extract. The least content was reported in petroleum ether extract. (Table 2)

Table 2. Total phenolic content of various extracts of stem bark of BV

Extract	Total Phenolic Content* (µg/mg of extract -GAE)
Petroleum Ether	15.91 ± 0.20
Ethyl Acetate	41.42 ± 1.63
Methanol	88.71 ± 1.04
Aqueous	50.75 ± 0.26

*All trials were carried out in triplicate. The values are means of three replicates with standard deviations (mean ± S.D.; n = 3), $p < 0.05$.

Total flavonoid content

The methanol extract reported the highest content for flavonoid too (133 µg/mg of extract), then stood the ethyl acetate and aqueous extracts. Least value was obtained for petroleum ether extract. (Table 3)

Table 3. Total flavonoid content of various extracts of stem bark of BV

Extract	Total Flavonoid Content* (µg/mg of extract - CE)
Petroleum Ether	10.64 ± 2.01
Ethyl Acetate	120.06 ± 1.91
Methanol	132.89 ± 3.34
Aqueous	92.31 ± 1.98

*All trials were carried out in triplicate. The values are means of three replicates with standard deviations (mean ± S.D.; n = 3), $p < 0.05$.

Estimation of tannins

The highest value was reported for the methanol extract followed by ethyl acetate, water and petroleum ether extracts. (Table 4)

Table 4. Total tannin content of various extracts of stem bark of BV

Extract	Total Tannin Content* (µg/mg of extract - TAE)
Petroleum Ether	17.08 ± 2.31
Ethyl Acetate	78.20 ± 5.27
Methanol	96.71 ± 6.45
Aqueous	67.48 ± 13.07

*All trials were carried out in triplicate. The values are means of three replicates with standard deviations (mean ± S.D.; n = 3), $p < 0.05$.

DISCUSSION:

Phenolics are important plant secondary metabolites with antioxidant activity owing to their redox potential, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides*[17]*. In previous studies the aqueous and ethanolic extracts of *B. variegata* L. have shown significant antioxidant activity *[18]*. The % free radical scavenging activity gradually increases with increasing concentrations of *B. variegata* extracts in DPPH radical scavenging assay. Dose dependent antioxidant activity pattern was also observed in phosphomolybdate assay. Antioxidant activity was directly correlated with the amount of total phenolic contents in the extracts *[17]*. *B. variegata* Linn. shows antihyperlipidemic activity by virtue of its antioxidant potential *[19]*. The flavonoids also account for the anti-inflammatory effects. Moreover**,** increase in enzymatic antioxidant (superoxide dismutase and catalase) levels accounts for the significant chemopreventive and cytotoxic effects of *B. variegate [20]*. These findings indicate that free radical scavenging in part has immense value in the prevention and treatment of deadly diseases and holds good only if the plant contains phenolics in an appreciable amount so that the plant can be commercially exploited. The results obtained a high yield of total phenolics from the stem bark pointing that it can be utilised as a remarkable source for the preparation of not only neutraceuticals as potent antioxidants but also for the treatment of other major health problems. Approaches can be made to

identify the individual polyphenolic compounds that are responsible for various pharmacological effects.

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