Pharmacognostical and Phytochemical Evaluation of Leaves of Bauhinia variegata Linn.

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ABSTRACT

Bauhinia variegata Linn. (Caesalpiniaceae) syn: kovidara and kachnar is a medium sized deciduous tree generally found in sub Himalayan region of India. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments. The present work was undertaken to establish the pharmacognostic and phytochemical standards along with HPTLC densitometric analysis of leaves for evaluating the plant material. The macro and microscopical studies indicated presence of pulvinus base with grooved petiolate leaf, emarginated apex, rough surface with 11-13 reticulate and palmate-divergent venation with scattered prismatic calcium oxalate crystals throughout the transverse section. Physiochemical studies revealed that total ash is 9.42%, acid insoluble ash is 5.72%, water-soluble extractive value is 3.30% and loss on drying at 105 °C is 6.27%. Preliminary phytochemical analysis revealed the presence of alkaloid, tannin, flavonoid, steroid, triterpenoid and saponin in different extracts. HPTLC fingerprinting for flavonoids revealed presence of two flavonoids rutin and kaempferol The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Key words: Bauhinia variegata, Leaf microscopy, Physico-chemical constants, Phytochemical, HPTLC finger print

INTRODUCTION

About 250 species of Bauhinia grow in the tropical regions of the world. It includes shrubs, trees and vines that are frequently planted for their showy flowers and ornamental foliage.[1] Bauhinia variegata Linn. is native to south eastern Asia and grows throughout India and China. It is most commonly cultivated in India.[2] Bauhinia variegata Linn. (Caesalpiniaceae) is a medium sized deciduous tree, known as (Sanskrit) kanchanara, (Hindi) kovidara and (Marathi) raktakanchar. Almost all parts are used in traditional medicine for the treatment of various ailments like asthma, ulcer, leprosy, piles, snake bite and liver complaints[3] and its extracts have been found to have antibacterial and antifungal activity.[4] It is also used in fever, diarrhoea, dysentery, hemorrhoids, piles, edema, skin diseases, wound healing, obesity, stomatitis, dyspepsia, flatulence and as tonic, astringent, laxative, anthelmintic, antileptic, antitumor, and carminative.[5] The leaves of other Bauhinia species are reported to have antiophidian,[6] antidiabetic,[7] antimalarial,[8] and antioxidant potential.[9]

Previously reported phytochemical constituents from the plant are lupeol, β-sitosterol, tannins, kaempferol-3-glucoside,[10] amides, carbohydrates, reducing sugars, crude protein, vitamin C, fibers,[11] calcium, phosphorus,[12] rutin, quercetin, quercitrin, apigenin, apigenin-7-O-glucoside,[13] dotetracont-15-en-9-ol and heptatriacontan-12,13-diol.[14] Inspite of its abundant uses, the phytochemical standards of Bauhinia variegata leaves have not been reported.

The present investigation deals with the qualitative and quantitative pharmacognostical and phytochemical evaluation of the leaf material.
MATERIALS AND METHODS

Plant Material
The plant material was collected from the natural park of the Mehsana district, Gujarat, India in the month of February 2009. The plant was authenticated by Pro. Y. B. Dabgar, Head, Department of Botany, Shri C. L. Parikh & R. R. Mehta Science College, Palanpur, Gujarat, India and a voucher specimen (No. SSPC/COG/05/2009) was deposited in Department of Pharmacognosy, Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India.

Pharmacognostical studies

Morphology
Morphological studies such as shape, size, apex, surface, base, margin, venation, taste and odour of leaves were carried out.

Microscopy
Microscopical studies were carried out using Nicon Labphot-2 instrument (Japan). The transverse sections with average thickness of 10-12 µm were taken with the help of rotary microtome. Dewaxing of the sections was performed by customary procedure. The section was stained with toluidine blue as per the method. Since, toluidine blue is a polychromatic stain, it rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies. Wherever necessary, sections were also stained with saffranin, fast-green and iodine potassium iodide reagents (for starch).

As a part of quantitative microscopy, stomatal number, stomatal index, vein islet number and vein termination number were studied by taking paradermal sections as well as clearing of leaf with 5% sodium hydroxide and epidermal peeling off by partial maceration employing Jeffrey’s maceration fluid as shown in table 1.

Physico-chemical constants
Physicochemical constants of the leaf such as the total ash, acid insoluble ash, water soluble ash and loss on drying were calculated based upon standard procedures.

Phytochemical analysis
For preliminary phytochemical studies, 300 g of powdered material was extracted in soxhlet apparatus with petroleum ether, chloroform, methanol and water. Extracts were dried in rotary evaporator and weighed. The extractive values for each extract is shown in table 2. The presence of various phytoconstituents like steroids and triterpenoids (Liberman and Buchard test), alkaloids (Dragendorff’s test), tannin (Ferric chloride test), flavonoid (Shinoda test), Sugar (Fehling solution test) were detected by usual method prescribed in standard text.

Densitometric HPTLC analysis for flavonoids
A densitometric HPTLC analysis was performed for the development of characteristic fingerprinting profile. 50% hydroalcoholic and methanolic extract of Bauhinia variegata Linn. leaves were dissolved with HPLC grade methanol 10 mg/ml. The solutions were sonicated for 10 min and used for HPTLC analysis. Then, 10 µl of the samples were loaded as 5 mm band length in the 10 × 10 Silica gel 60F254 TLC plates using Hamilton 254 syringe and Desaga Sarstedt-gruppe AS 30 instrument. The sample loaded plate were kept in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phase along with 2000 µg/ml rutin and 200 µg/ml kaempferol standard solutions. The plates were developed in the Ethyl acetate-Butanol-Formic acid-Water (10:6:2:2 v/v/v/v) for rutin (Rf 0.48) and Toluene-Ethyl acetate-Methanol-Formic acid (6:3:0.2:0.4 v/v/v/v) (Rf 0.56) for kaempferol up to 90 mm. The developed plate was dried using hot air to evaporate solvents from the plate and kept in Photo-documentation chamber (Desaga) and captured the images at UV254. Finally, the plates were fixed in scanner stage and scanned at 380 nm and 254 nm for rutin and kaempferol respectively.

Table 1: Leaf constants of the Bauhinia variegata Linn. leaf

<table>
<thead>
<tr>
<th>Leaf constant</th>
<th>Values</th>
</tr>
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<tbody>
<tr>
<td>Palisade ratio</td>
<td>4.8</td>
</tr>
<tr>
<td>Stomatal index</td>
<td>5.6</td>
</tr>
<tr>
<td>Vein-islet number</td>
<td>5.8</td>
</tr>
<tr>
<td>Vein termination number</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Table 2: Extractive values of the Bauhinia variegata Linn. leaf

<table>
<thead>
<tr>
<th>Extract</th>
<th>Colour</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>Yellowish brown</td>
<td>3.41</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Brownish black</td>
<td>1.72</td>
</tr>
<tr>
<td>Methanol</td>
<td>Brown</td>
<td>8.76</td>
</tr>
<tr>
<td>Aqueous</td>
<td>Greenish brown</td>
<td>7.25</td>
</tr>
</tbody>
</table>

Figure 1: Leaf of Bauhinia variegata Linn.
RESULTS AND DISCUSSION

Organoleptic characters
Colour of Bauhinia variegata Linn. leaves is green on both side when fresh and brown in dry state. Size and shape is 13-15 × 12-14 cm, long as broad as or rather broader than long, cleft 1/4 to 1/3 of the way down into 2 obtuse lobes, pulvinus base with grooved petiolate, linear-lanceolate with entire margin with soft stipules. Apex of the leaf is broad and emarginated. The surface of leaf is rough surface with 11-13 reticulate, palmate-divergent venation as shown in figure 1. Taste is slightly bitter and having weak odor.

Microscopy
Transverse section of the leaf (Figure 2)
Lamina
It shows dorsi-ventral nature; more densely covered upper epidermis with cuticle than lower epidermis and made up of thin walled tangentially elongated rectangular cells. Mesophyll in the lamina shows the presence of 2-3 layers of palisade parenchyma below the upper epidermis and spongy parenchyma above the lower epidermis as shown in figure 2 (B). Scattered prismatic calcium oxalate crystals are present throughout the mesophyll.

Midrib
Ventral side of the midrib is slightly concave. Shape of the middle vein portion in the TS is oblong but elongated tapered at ventral side and also shows the irregularities on its lower epidermis. Midrib contains ‘U’- shape well developed vascular bundle at the centre surrounded by sclerenchyma (pericyclic lignified fibrous tissue in a band). Vascular bundle shows the presence of the xylem at the upper side and phloem at the lower side and well developed collenchyma below the upper epidermis and above the lower epidermis with scattered prismatic calcium oxalate crystals.

TS of the leaf also show the presence of unicellular, 3-5 celled multicellular uniseriate- cuticlerised covering trichomes as well as unicellular sessile and unicellular head with unicellular stalked glandular trichomes. Trichomes are more prominent on the lower epidermis than upper epidermis.

Powder microscopy
The powder microscopy showed the presence of 142.8-199.92 µm long multicellular uniseriate trichomes, 57.12 µm in diameter prismatic calcium oxalate crystals, lignified annular and spiral xylem vessels, portion of epidermal cells with anomocytic stomata as shown in figure 3.

Figure 2: Leaf structure of Bauhinia variegata Linn.

Figure 3: Powder microscopy of leaves of Bauhinia variegata Linn.
Quantification of rutin and kaempferol in both extracts shows that the sample of 50% hydroalcoholic extract (1.114 mg/100 g) contains more rutin than the sample from the methanolic extract (0.075 mg/100 g) whereas the sample of 50% hydroalcoholic extract (0.193 mg/100 g) contains more kaempferol than the sample from the methanolic extract (0.014 mg/100 g).

CONCLUSION

A variety of standardization parameters like morphological, microscopic, physico-chemical, phytochemical and chromatographic characterization were studied and generated
data could be useful for the assessment of quality of plant material, and also to check the adulteration and substitution etc., for future reference.

The pharmacognostic study of Bauhinia variegata Linn., have furnished a set of qualitative and quantitative standards that may substantiate to ascertain it’s identity and to establish the quality and purity of this plant material in closely related species. Densitometric HPTLC analysis may serve a supplementary data for the standardization of the drug, particularly of different batches. This could also serve in the establishing data for preparation of monograph of this plant.

ACKNOWLEDGEMENT

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