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RESEARCH ARTICLE

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES ON THE LEAF EXTRACTS OF *Bauhinia tomentosa* Linn

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ABSTRACT

Bauhinia tomentosa Linn., leaves were selected to screen pharmacognostic and phytochemical studies. Plant material was collected from a garden in Thiruvarur district, Tamilnadu, India. *Bauhinia tomentosa Linn.*, is a well-known medicinal plant, which has been valued in ancient systems of medicine for the treatment of gastrointestinal infections. The leaf extracts of various solvents were subjected to pharmacognostical and phytochemical analysis. Variable fluorescence nature of the plant was also noted against day and UV light. Leaf extracts contain alkaloids, steroids, terpenoids, flavonoids, saponins, phenolic compounds, tannins, cardiac glycosides etc., which could be a reason for the plants pharmacological activity. These observations would be of great value in the authentification of this plant in its crude form.

Keywords: *Bauhinia tomentosa* leaves, pharmacognosy, fluorescence analysis, phytochemical screening,

INTRODUCTION

Bauhinia tomentosa Linn., belongs to the family Caesalpiniaceae, is an erect shrub, found in the plains southwards of Delhi, in the peninsular region and in West Bengal. Leaves broader than long, coriaceous, pubscent below; flowers with a distinct odour, usually axillary, pendant and half open, yellow with a maroon dot at the base of the central petal; pods distinctly stalked, glabrous, 6-10 seeded. It is often cultivated in gardens for its fragrant and pretty yellow flowers. A decoction of the root bark is prescribed for liver troubles and as a vermifuge. Infusion of the stem bark is useful as an astringent gargle. The leaves constitute an ingredient of a plaster applied to abscesses. They are used as a yellow dye along with turmeric. The dried leaves, buds and flowers are used in dysentery. The fruit is diuretic. The seeds are used as tonic and yield a fatty oil called Ebony Oil [1]. Seeds are eaten for their aphrodisiac action and made into a paste with vinegar as an efficacious application to wounds inflicted by poisonous animals, snakes and scorpions. Bruised bark ground with rice-water into a paste is externally applied to tumours and wounds such as scrofulous [2].

The present study deals with the pharmacognostical and phytochemical screening of *Bauhinia tomentosa* Linn., leaves.

MATERIALS AND METHODS

Plant material

The leaves of *Bauhinia tomentosa* Linn., were collected from a garden in Perumpannaiyur, Thiruvarur district, Tamil Nadu and were for the purpose of extraction. The organoleptic characters of the leaves were studied.

Pharmacognostical studies

Preparation of plant extract

The leaves of **Bauhinia** *tomentosa* Linn., were first washed, shade dried and then milled into coarse powder using a mechanical grinder. 50 g of the powdered leaf material was extracted with hexane, chloroform, acetone, ethanol and water successively by hot percolation method[3]. The percentage yields were shown in Table 2.

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Fluorescence Analysis

Fluorescence characteristics of the powdered drug with different chemicals were observed in day light and ultraviolet light at 254 nm. The powdered leaf was treated with various solvents like hexane, alcohol, chloroform, benzene, acetone and ethyl acetate and acids like 1N hydrochloric acid, 50% sulphuric acid and alkaline solutions like aqueous and alcoholic NaOH. Observations of fluorescence analysis were recorded at 0, 24 and 48 hours [3] .

Preliminary phytochemical screening

One gram of the acetone, ethanol and aqueous extracts of the leaves of *Bauhinia tomentosa* Linn., were dissolved in 100 ml of their own mother solvents to obtain a stock of concentration 1 % (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening following the methodology [3,4].

Screening procedure

1. Test for Alkaloids

i) Dragendorff's test

One ml of the extract was added with 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

ii) Mayer's test

One ml of the extract was added with 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

2. Test for steroids

Two ml of acetic anhydride was added with 0.5 g ethanolic extract of each sample with 2 ml of H_2S0_4. The color changed from violet to blue or green in some samples indicates the presence of steroids.

3. Test for terpenoids

Salkowski test

0.5 g of the extract was added with 2ml of chloroform was and 3 ml of concentrated H_2S0_4 was also carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids. This type of reaction was observed and recorded.

4. Test for flavonoids

i) Alkaline reagent test

Few drops of dilute ammonia were added to a portion of the leaf extract and concentrated HCl was also added. A yellow colouration indicated the presence of flavonoids. The reaction was observed and recorded.

ii) Zinc Hydrochloride test

Few drops of extract were added with zinc dust and concentrated HCL. The presence of red colouration indicates the presence of flavonoids.

iii) Aluminium test

Few drops of the extract was added with 1% aluminium solution was added. Formation of yellow colour indicated the presence of flavonoids. The type of reaction was observed and recorded.

5. Test for Saponins:

Two gram of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously until the formation of a stable persistent froth. The frothing was mixed with 3 drops of olive oil and again shaken vigorously, and observed for the formation of emulsion.

6. Test for tannins

i) Lead acetate test: A little quantity of the test solution was mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

ii) One ml of the extract was added with ferric chloride solution. Formation of a blue black or brownish green color product shows the presence of tannins.

iii) A little quantity of the extract was treated with aqueous ammonia solution. A deep green color indicates the presence of tannins and the type of reaction was observed and recorded.

7. Test for phlobatannins

The aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate indicates the presence of phlobatannins.

8. Test for cardiac glycosides

Keller-Killiani test

0.5 g of extract was diluted with 5 ml water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar, characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer.

9. Test for Proteins

Xanthoprotein test

One ml of the extract was added with 1ml of concentrated nitric acid. A white precipitate is formed, and it is boiled and cooled. To that 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.

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10. Test for carbohydrates

Two ml of the extract was added with 1ml of Barfoed's reagent and boiled. Reddish brown precipitates indicate the presence of carbohydrates.

11. Test for Aminoacids

Ninhydrin Test

Three ml of test solution was added with 3 drops of 5% ninhydrin solution in a test tube and heated in boiling water bath for 10 minutes. Formation of purple or bluish color indicates the presence of amino acids.

12. Test for reducing sugars

i) Fehling's test

One ml of the extract was added with equal quantities of Fehlings solution A and B and upon heating, formation of a brick red precipitate indicates the presence of sugars.

ii) Benedict's test

Five ml of Benedict's reagent was added with 1 ml of extract solution and boiled for 2 minutes and cooled. Formation of red precipitate was showed the presence of sugars.

A few ml of extract was treated with saffranine solution. Pink colour formation indicates the presence of lignin.

14. Test for inulin:

A few ml of the extract was added with 1 ml solution of α-naphthol and 0.5 ml sulphuric acid. Formation of brownish red colour indicates the presence of inulin.

RESULTS AND DISCUSSIONS

Good and effective raw material is needed to maintain effective therapeutic potentials of medicinal plants. Moderate pharmacognostical features is noted from different geographic locations. Standardization is needed to maintain the quality of raw materials, used as medicines. In the present study, pharmacognostical, phytochemical and fluorescent features of the plant *Bauhinia tomentosa* Linn. leaves were analysed and reported. Morphological evaluation revealed the shape of the selected leaf. *Bauhinia tomentosa* Linn. leaves are 4 to 7 cm long, about as wide, and split about one-third to the base into two, with oval, rounded lobes. The colour of the leaf was green. Leaf powder was found to be mild tamarind odour and with bitter taste (Table -1). Texture of the powder used for the study was coarse and the fracture was smooth (Table 1).

13. Test for Lignin

In the present study much higher extractive values were noted in aqueous extract (9.7 %) whereas lower extractive values were noted for chloroform (0.4%) and acetone extract (7.1%). Higher extractive matters were collected using water (9.7 %) followed by ethanol (8.6 %) (Table – 2).

Table 1: Organoleptic characters of *Bauhinia tomentosa* Linn leaves

S. No	Character	Observation
1	Color	Green
2	Odour	Mild tamarind
3	Taste	Bitter
4	Texture	Coarse
5	Fracture	Smooth

Table 2: Extraction values of different extracts of *Bauhinia tomentosa* leaves

S. No	Extracts	% Yield (w/w)
1	Hexane	4.5%
2	Chloroform	0.4%
3	Acetone	7.1%
4	Ethanol	8.6%
5	Water	9.7%

Red coloured **c**hromophore was produced when the leaf powder was treated with chloroform, benzene, 1N HCL under day light observations at 0-24 hours. Red and dark colours were noted at 48 hours, when the leaf powder was treated with benzene, aqueous NaOH and 1N HCL. Black colour was noted when the powder was treated with 50% sulphuric acid (Table 3). Leaf powder of *Bauhinia tomentosa* exhibited different coloured chromatophores (Orange, Yellow, Majenta, Sandal, Greenish yellow, Red) when treated with different chemicals and observed under UV and day light at different intervals (Table – 3).

Phytochemical screening of the acetone, ethanol and aqueous extracts of the leaves of *Bauhinia tomentosa* Linn. showed the presence of steroids, alkaloids, terpenoids, flavonoids, saponins, phenolic compounds, tannins, lignin, fat and oil, inulin, cardiac glycosides, proteins, carbohydrates, aminoacids, reducing sugars and absence of phlobatannins. Acetone extracts showed the presence of steroids, flavonoids and carbohydrates. Aqueous extracts showed the absence of alkaloids, tannins and the ethanolic extracts showed the absence of saponins and cardiac glycosides (Table 4).

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Table 3: Fluorescence analysis of different extracts of *Bauhinia tomentosa* Linn., *leaves*

S. No	Test	0 hours		24 hours		48 hours	
		Day light	UV light	Day light	UV light	Day liht	UV light
1	Chloroform	Red	Light Yellow	Orange	Greenish Yellow	Orange	Yellow
2	Hexane	Light Brown	Light Yellow	Light Brown	Greenish Yellow	Light Brown	Greenish Yellow
3	Benzene	Red	Yellowish Brown	Red	Brown	Red	Brown
4	Aqueous NaOH	Majenta	Brownish Green	Dark red	Dark Brown	Dark Red	Greenish Brown
5	Alcoholic NaOH	Dark red	Yellow	Dark red	Greenish Yellow	Dark red	Greenish Red
6	1 N Hcl	Pale Yellow	Yellow	Red	Yellowish Red	Red	Yellowish Red
7	Ethanol	Sandal	Whitish Yellow	Sandal	Greenish Yellow	Yellow	Greenish Yellow
8	Ethyl acetate	Pale Yellow	Pale Yellow	Pale yellow	Greenish Yellow	Yellow	Greenish Yellow
9	Acetone	Light Brown	Yellow	Dark Brown	Greenish Yellow	yellow	Greenish Yellow
10	50% Sulphuric acid	Pink	Pinkish Brown	Pink	Brown	Black	Dark Brown

Table 4: Qualitative phytochemical analysis of leaf extracts of *Bauhinia tomentosa* Linn.,

S.No	Test	Acetone extract	Ethanol extract	Aqueous Extract
1	Alkaloids	_	+	_
2	Steroids	+	+	+
3	Terpenoids	_	+	+
4	Flavonoids	+	+	+
5	Saponins	_	_	+
6	Phenolic compounds	_	+	+
7	Tannins	_	+	_
8	Lignin	_	+	+
9	Phlobatannins	_	_	_
10	Fat and Oil	_	+	+
11	Inulin	_	+	+
12	Cardiac glycosides	_	_	+
13	Proteins	_	+	+
14	Carbohydrates	+	+	+
15	Aminoacids	_	+	+
16	Reducing sugars	_	+	+

(+) indicates presence (-) indicates absence

Medicinal plants containing phytochemicals with various biological activities that can be of significant therapeutic index. Different phytochemicals have been found to possess a wide range of beneficial properties, which may help in

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protection against infectious diseases and disorders. Phytochemicals such as the steroids and saponins are responsible for the activities of the Central Nervous System [5].Steroids and triterpenoids shown to have analgesic properties. The terpenoids have shown to decrease blood sugar level in animal studies [6].The saponins possess hypocholesterolemic and antidiabetic properties [7]. It has been found that more highly oxidized phenols are more inhibitory to microorganisms [8,9]. Flavonoid compounds inhibit multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifranchside [10], glycyrrhizin from licorice[11] and chrysin [12]against HIV.Tannins received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, like green teas and red wines can cure or prevent a variety of ills [13].Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity and a wide range of anti-infective actions have assigned to tannins [14].Saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects [6,15]

Phytochemical screening of aqueous and ethanolic extracts of the leaves of *Bauhinia tomentosa* Linn revealed the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins and cardiac glycosides, possessing many protective properties including antimicrobial effects.

CONCLUSION

Pharmacognostical properties such as organoleptic and physicochemical characters and features exhibited under fluorescence analysis were worked out. Also the phytochemical screening process shows the presence of biologically active phytochemicals in the leaf extracts of *Bauhinia tomentosa* Linn. The medicinal properties of this plant may be due to the presence of phytochemicals like tannins, flavonoids, terpenoids and steroids.

REFERENCES

1. Anonymous, The wealth of India. CSIR, New Delhi, 1976, 2: 55
2. Nadkarni AK, Indian Materia Medica. Popular Prakashan Pvt Ltd., Tardeo, Mumbai 400 034, 1976, 1:183-184
3. Harborne JB Phytochemical methods, A guide to modern techniques of plant analysis. 3 rd edition, Chapman and Hall Int. Ed., New York, 1998
4. Kokate CK, Pharmacognosy, 16 th edition, Nirali Prakasham, Mumbai, India, 2001
5. Argal A, Pathak AK, CNS activity of *Calotropis gigantea* roots, *J. Ethnopharmacolog.*, 2006, 106 : 142 –145
6. Liu RH, Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals, *Am. J. Clin. Nutr.*, 2003, 78: 517S – 520S
7. Rupasinghe HP, Jackson CJ, Poysa V, Di Berado C, Bewley JD, Jenkinson J, Soyasapogenol A and B distribution in soyabean in relation to seed physiology, genetic variability and growing location, *J. Agri. Food Chem.*, 2003, 51: 5888 - 5894
8. Scalbert A, Antimicrobial properties of tannins, *Phytochemistry*, 1991, 30 : 3875 - 3883
9. Urs NVRR, Dunleavy JM, Enhancement of the bactericidal activity of a peroxidase system by phenolic compounds (*Xanthomonas phaseoli* var *sojensis*, soybeans), *Phytopathology*, 1975**,** 65 : 686 - 690
10. Pengsuparp TL, Cai HHS, Fong AD, Kinghorn JM, Mechanistic evaluation of new plant derived compounds that inhibit HIV-1 reverse transcriptase, *J. Nat. Prod.*,1995, 58 :1024 -1031
11. Watanbe H, Miyaji C, Makino M, Abo T, Therapeutic effects of glycyrrhizin in mice infected with LP-BMS murine retrovirus and mechanisms involved in the prevention of disease progression, *Biotherapy*, 1996, 9 : 209 - 220.
12. Critchfield JW, Butera ST Folks TM, Inhibition of HIV activation in latently infected cells by flavonoid compounds, AIDS Res. Hum. Retroviruses, 1996, **12**:39
13. Serafini M, Ghiselli A, Ferro-Luzzi A, Red wine, tea and antioxidants, Lancet, 1994, 344 : 626
14. Haslam E, Natural polyphenols (vegetable tannins) as drugs: possible modes of action, *J. Nat. Prod.*, 1996, **59**:205-215
15. Akindele AJ, Adeyemi OO, Anti inflammatory activity of the aqueous leaf extracts of Byrsocarpus coccineus, *Filoterapia*, 2007,78: 25 -28

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