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Pharmacognstic standardization and preliminary phytochemical studies of *Bauhinia acuminta*

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Abstract

The present study help to authenticate the medicinally important plant *B. acuminata*. Qualitative and quantitative features may be helpful for establishing the pharmacopeia standards. Morphology as well as various pharmacognostic aspects of different parts of the plant were studied and described along with phytochemical and physicochemical parameters, which could be helpful in further isolation and purification of medicinally important compounds.

Keywords: Pharmacognosy, phytochemical, B. acuminata

Introduction

Pharmacognosy is a systematic science which deals with therapeutic efficacy of herbal drugs. It depends upon the quality and quantity of the active compounds obtained from the crude drugs of plant of natural origin ^[1]. Pharmacognosy is the concurrent application of various precise disciplines with the object of acquiring knowledge of drugs from every point of view. Traditional medicine is also a part of pharmacognosy and most of the third world countries still depend on the use of herbal medicine ^[2]. The World Health Organization (WHO) defines traditional medicine (TM) as the total combination of knowledge and practices, where explicable or not, used in diagnosing, preventing or eliminating physical, mental or social diseases which may depend exclusively on experience and observation handed down from generation to generation, verbally or in writing It provides the scientifically important information about the intent, purity and quality of the plant drugs ^[3].

Bauhinia acuminata of the family Caesalpiniaceae is a pantropical genus of 300 species distributed throughout the tropical regions of the world and native to tropical Southeast Asia. This plant is reported as medicinally important in traditional system of medicine and are used extensively for the treatment of inflammation, headache, fever, tumors, skin infections etc ^[4]. It is an evergreen large flowering shrub. The bark, flower and root of *B. acuminata* are used for various skin diseases, worms, tumours and diabetes. The bark and leaves of *B. acuminata* are used to treat biliousness, a remedy recommended by the Indian Vaiydas ^[5]. Moreover, the leaf of *B. acuminata* is used to treat bladder stone, venereal diseases, leprosy, asthma and digestive diseases .Previous researches on this drug proved that it is having antioxidant action, free radical scavenging action, diuretic action, hypoglycemic action, anti-cancer action, anti-inflammatory action ^[6].

Materials and Methods

Plant collection, Identification and Preparation

The leaves of *Bauhinia acuminata* L. (Fabaceae) were collected during August – September 2015 Angamaly, Ernakulum Kerala. The plant was identified and authenticated by Dr P. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai. After authentication, one plant sample was deposited as a voucher specimen in cancer biology lab, Loyola college, Chennai. The fresh leaves were collected in bulk from young matured plant; they were washed with running tap water, followed by distilled water, air dried in shade, then homogenized to a fine powder. The powder of the leaf was stored in air-tight containers and labeled properly until further use.

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Bauhinia acuminata L.

Kingdom	:	Plantae
Division	:	Magnoliophyta

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Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Genus	:	Bauhinia
Species	:	B. acuminata

Macroscopic studies

The macroscopy of leaf of *Bauhinia acuminata* species was described with the help of Floras

Pharmacognostic Studies

Physicochemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed in Indian Pharmacopeia, 1996 and WHO guidelines on quality control methods for medicinal plant material ^[7]

Determination of Moisture Content or Loss on Drying

About 6 g of each raw material was accurately weighed and was taken in a previously dried and tarred flat weighting bottle in IR moisture balance. The temperature was adjusted to 105 $^{\circ}$ C and heating was done for 5 minutes. The procedure was repeated three times for different samples and the loss in weight of the raw material was calculated with respect to the original weight.

The formula used for calculating LOD was= W1/W2 x100

W1-weight of raw material after heating W2- Original weight of the raw material

Extractive values

Determination of Chloroform Soluble Extractives

Exactly weighed 6 g of air dried coarse powder was macerated with 100 ml of chloroform in a glass-stoppered conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. About 25ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105° C for 6 hours, cooled in a desiccator for 30 minutes and weighed immediately.

Determination of Water Soluble Extractives

6 g of air dried coarse powder was macerated with 100 ml of water in a glass-stoppered conical flask with frequent shaking for 4 to 6 hours and kept aside for 18 hours. Thereafter it was filtered rapidly taking care against loss of solvent. About 25ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105° C for 6 hours, cooled in a desiccator for 30 minutes and weighed. The percentage w/w of water soluble extractive was calculated with reference to the air-dried drug.

Determination of Ash Values

The determination of ash values is meant for detecting lowgrade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a means of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash ^[8]. Ash values such as total ash, acid insoluble ash, and water soluble ash, acid soluble and sulfated ash were determined. The total ash determination method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological ash", which is the residue of extraneous matter adhering to the plant surface.

Determination of Total Ash

About 4 g powder was accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as afine layer at the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Determination of Acid Insoluble Ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of Water Soluble Ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on a filter paper and washed with hot water and transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried parts.

Determination of Acid Soluble Ash

Total ash treated with dilute hydrochloric acid reacts with minerals to form soluble salts and the insoluble residue consists mainly of silica, as acid insoluble ash. To the crucible/silica dish containing the total ash obtained by the previous test, 25ml of HCl (N70g/l) was added, covered with a watch glass and boiled gently for 5 min on a hot plate or burner. Watch glass was rinsed with 5ml of hot water and washings were added to the crucible. Insoluble matter was collected on a filter paper by filtration and rinsed repeatedly with hot water until the filtrate was found to be neutral/free from acid. Filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450 -500 C. Silica dish was cooled in desiccator for 30 min and weighed without delay. Content of acid insoluble ash as% was calculated with reference to the air-dried ash.

Acid insoluble ash % =
$$\frac{(B-C)}{A}$$
 100

Where, A = sample weight in g B = wt. of dish + contents after drying (g) C = wt. in g. of empty dish

Determination of Sulphated ash

A silica crucible was heated to red for 10 min. and was allowed to cool in a desiccator and weighed. A gram of ash was accurately weighed and transferred to the crucible. It was ignited gently at first, until the ash was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes were no longer evolved and ignited at 800 °C \pm 25 °C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool and add few drops of concentrated sulfuric

acid were added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

Phytochemical Analysis

Qualitative analysis of Phytochemicals from the EA fraction of CEBA

All reagents were obtained from Hi-Media Pvt Ltd, Mumbai, India and were of analytical grade.

Detection for Carbohydrates

0.5 gm. of the *B. acuminta* was dissolved in 5 ml of distilled water and filtered. To the extract two drops of Molisch's reagent and two drops of concentrated H_2SO_4 were added, Formation of purple-violet ring indicated the presence of carbohydrates.

Detection of Glycosides

0.5 gm of the *B. acuminta* was hydrolyzed with 20 ml of HCl (0.1N) and filtered. To the filtrate, few drops of glacial acetic acid and one drop of 5% FeCl₃ and concentrated H_2SO_4 were added, Formation of reddish brown colour at the junction of two liquid layers and upper layer turning bluish green indicated the presence of glycosides

Detection of Saponins

0.5gm of *B. acuminta* was diluted to make up to 20 ml with distilled water and slowly shakes in a graduated cylinder for 15 minutes. 1 cm layer of foam shows the presence of saponins.

Detection of Alkaloids

0.5 gm of *B*.*acuminta* extract was dissolved in 10 ml of dilute HCL (0.1N) and filtered.

a. Mayer's test

Filtrate was treated with Meyer's reagent; formation of yellow cream-colored precipitate indicated the presence of alkaloids.

b. Dragendrodroff's test

Dragendrodroff's reagent: 8 gm of bismuth subnitrate was dissolved in 20 ml of nitric acid.

a.27.2 gm. of Potassium iodide was dissolved in 50 ml of distilled water; (a) and (b) were mixed and the volume was adjusted to 100 ml with distilled water. The two solutions were mixed together, and the filtrate was treated with Dragendrodroff's reagent; formation of red colored precipitate indicated the detection of alkaloids.

Detection of Flavonoids

To 100 mg of *B. acuminta* few drops of NaOH solution were added in a test tube. Formation of intense yellow color that became colorless on addition of few drops of dilute HCl indicated the presence of Flavonoids.

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Detection of Phenolics and Tannins

100 mg of *B. acuminta* was boiled with 1 ml of distilled water and filtered. The filtrate was used for the following test,

a. Ferric chloride test: To 2 ml of filtrate, 2 ml of 1% ferric chloride solution was added in a test tube. Formation of bluish black color indicated the presence of phenolic nucleus

b. Test for Tannins: To the extract 0.5 ml NaOH was added; Formation of precipitate indicated the presence of tannins.

Detection of Phytosterols and Triterpenoids

0.5 gm. of was treated with 10 ml chloroform and filtered. The filtrate was used to test the presence of Phytosterols and Triterpenoids

a. Liebermann's test: To 2 ml of filtrate in hot alcohol, few drops of acetic anhydride was added. Formation of brown precipitate indicated the presence of sterols

b. Leiberman-Bucharat test: To the extract, few drops of acetic acid and concentrated H₂SO4 were added; deep red ring at the junction of two layers indicated the presence of triterpenes.

c. Salkowaski test: To the extract solution few drops of conc. Sulphuric acid were added and shaken and allowed to stand, lower layer turned red indicating the presence of sterols.

Detection of Fixed Oils and Fats

Oily Spot test: One drop of extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicated the presence of fixed oil

Results

Microscopy

Microscopy shows normal structures of root, stem and leaves.

Pharmagnostic analysis

Physicochemical investigations of *B. acuminata* related to the moisture content, total ash, acid insoluble ash, acid soluble ash, water soluble and sulphated ash, extractive values were carried out. Extractive values of chloroform and aqueous extract were 22.40 and 20.64 respectively. The moisture content was 7.2% (Table 4). Physicochemical investigation of *B. acuminata* showed the total ash, acid insoluble ash, acid soluble ash, water soluble and sulphated ash extractive values were 6.6% w/w, 5.33% w/w and1.33% w/w, 5.33% w/w 2.75) respectively (Table.5).The percentage yields of extracts in methanol, petroleum ether, chloroform and ethyl acetate were 12%, 4%, 2.8% and 2% respectively (Table 6).

Table 1: Extractive values and moisture content of B. acuminata

	Chloroform extract	Aqueous extract	Moisture content
B. acuminata	(%)	(%)	(%)
	22.40	20.64	7.2

 Table 2: Total Ash, Acid Insoluble, Acid Soluble, Water Soluble

 and Sulphated Ash of B. acuminata

B. acuminata	Ash value (%)
Total ash (%)	6.66
Acid insoluble ash (%)	5.33
Acid soluble ash (%)	1.33
Water Soluble ash (%)	5.3
Sulphated ash (%)	2.75

Table 3: Extractive values of different solvents of *B. acuminate*

Solvents	Percentage of Extract (W/w%)
Petroleum ether	4
Chloroform	2.8
Ethyl acetate	2
Methanol	12

Table 4: Phytochemicals present in the B. acuminata

Tests		R Acuminata
Test for carbohydrate	Formation of purple-	+
Molisch's test	violet ring	
Test for Glycosides		
Keller-Killiani test	bluish green	+
Test for alkaloids		
a. Mayer's test.		+
b. Dragendrodroffs test		+
Test for Flavonoids		
Alkaline reagent test	Intense yellow color that	+
Test for Phenolic		+
a. Ferric chloride test		
Test for Triterpenoids		
a. Salkowaski test		+
Test for fixed oils and fats		
Oily spot test		+

Discussion

Pharmacognstic study of B. acuminata ensures the quality control and authenticity of a future drug from the crushed or powdered plant materials. The physiochemical parameters serve as standard data for the quality control of the preparations containing this plant in future ^[9]. Moisture is one of the major factors responsible for the deterioration of drugs and herbal formulations. The moisture promotes the degradation processes caused by enzymes, development of microorganism's oxidation and hydrolysis reactions. The study recorded moisture content of B. acuminata leaf sample as 7.2%, which is found to be greater than the normal water content (14%) of other medicinal plants. (Table 1) (www.intechopen.com). Extractive values of chloroform and aqueous extracts of B. acuminata were 22.40 and 20.64 respectively, it ensures the quality as well the purity of the plant.

Physicochemical investigations of *B. acuminata* showed the total ash, acid insoluble ash, acid soluble ash, water soluble and sulphated ash extractive values of 6.6% w/w, 5.33% w/w and 1.33% w/w, 5.33% w/w 2.75 respectively .The resulted values were significantly lesser than the maximum acceptable limit of 14% recommended by European Pharmacopoeia. Both the plant tissue (physiological ash) and other extraneous matter (e.g. soil and sand) adhering to the drug surface (non - physiological ash) can also produce ash. The ash values, alcohol soluble, chloroform and water soluble extractive values of leaves were determined as per the Indian Pharmacopoeia method ^[10]. Physicochemical parameters of total ash, acid soluble ash, sulphated ash and acid insoluble

ash in the leaves of *B. acuminata* are highly in accordance with previous research reports in *B. purpurea*, *B. variegata*, *B. racemosa*^[11, 12].

Water soluble extracts of *B. acuminata* leaves were 18.4% (W/W) and the values were not less than 15% (w/w) as stipulated by the European Pharmacopoeia ^[13]. The alcohol soluble extract value of *B. acuminata* was 8%. This could help to detect exhausted and already utilized drug which could be fraudulently used as substitutes and adulterants ^[14]. The results suggested that the powdered plants have high soluble extractive value in methanol followed by petroleum ether.

The phytochemical research approach is considered effective in discovering bioactive profile of plants of therapeutic importance. During the present study *B.acuminata* were subjected to qualitative phytochemical screening of various phytochemicals by adopting standard methodology. Thus, extracts of *B. acuminata* was screened for the presence and absence of various phytochemical constituents. Extracts showed the presence of saponins, oils and fats, carbohydrates, phenols tannins and flavonoids (Table 4).

Phytochemicals have always been sought after because of their inherent potential to cure disease, as demonstrated by ancient medicinal practices. They selectively kill rapidly dividing cells, target abnormally expressed molecular factors, eliminate oxidative stress, control cell growth factors, prevent angiogenesis of cancerous tissue and induce apoptosis ^[15]. The genus *Bauhinia* has been studied extensively in recent years for its medicinal values. Phytochemical investigations of the *Bauhinia* genus have revealed the presence of a number of compounds including steroids, glycosides, triterpenes, lactones, and flavonoids ^[16].

Preliminary phytochemical screening of the B. acuminata ascertains the presence of flavonoids, triterpenoids, carbohydrates, glycosides, oils and fats. These are the active biological constituents which are responsible for different pharmacological actions of B. acuminata. Flavonoids are polyphenol compounds. They have anti-oxidative, antianti-mutagenic inflammatory, and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function [17, 18]. One study proved that flavonoids from plant resources may be inversely associated with adenocarcinomic human alveolar basal epithelia ^[19]. k. Terpenoids possess antimicrobial ^[20] antioxidant ^[21] antiinflammatory ^[22] and anticancer activities ^[23, 24] Glycosides have a long history of therapeutic applications. They can induce apoptosis and inhibit the growth of cancer cell lines at low concentrations. High-throughput screenings of drug libraries have therefore identified cardiac glycosides as potent inhibitors of cancer cell growth [25, 26].

Carbohydrate is reported to have numerous roles in living systems, such as the storage and transport of energy (starch, glycogen) and structural components (cellulose in plants, chitin in animals. In addition, carbohydrates and their derivatives play major role in the working process of the immune system, fertilization, pathogenesis, blood clotting and development ^[27]. Previous studies on the phytochemical screening of *B. acuminata* revealed the presence of alkaloids, flavonoids, starch, tannins, reducing sugars, phenolic compounds, amino acids and lignin, saponins, oils and fats in leaves and stems of *B acuminata* ^[28].

Conclusion

Preliminary pharmacognostical and phytochemical analysis of *B. acuminta* Linn Was conducted. The various parameters studied are useful to identify and authenticate the traditionally important medicinal plant *B. acuminta*. WHO emphasized to conduct such studies which ultimately are helpful in the preparation of herbal monographs and pharmacopeia standards. The phytochemical studies indicate that it is a useful plant to investigate for phytochemical and biological assays. Further this study will also be helpful to minimize the chances of adulteration of this useful drug when the drugs are available in the powdered form.

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