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Physicochemical, Qualitative and Quantitative Phytochemical Analysis of the Leaf and Bark of *Bombax Ceiba* L (Red Silk Cotton Tree)

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ABSTRACT

Bombax ceiba L is a big deciduous tree found in tropical and subtropical regions of Asia, Africa and Australia. Conventional systems of medicine such as Ayurveda, Siddha and Unani have been highlighted the use of *B. ceiba* parts (bark, leaves and flower) for the treatment of manydiseases like hypertension, HIV infections, inflammation, catarrhal affection, ulcer, acne, gynecological disorders, fever, dysentery, algesia, hepatotoxicity, piles and urinary infections. The aim of the present study was to evaluate pharmacognostic, physicochemical, qualitative and quantitative phytochemical analysis of leaf and bark of *B. ceiba* collected from Bhopal region of Madhya Pradesh. The pharmacognostic studies out in terms of various investigations like organoleptic or morphological characters, microscopic or anatomical studies, physicochemical evaluations (loss on drying, ash value, extractive values and acid insoluble ash value). Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. The detail microscopy of bark revealed the presence of cork cell, lignified fibre, calcium oxalate crystals, xylem vessels. Physiochemical parameters such as percentage of foreign matters, ash values, loss on drying swelling index, extractive values were determined. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, alkaloids and glycosides. In the procedure of quantitative analysis of flavonoids and phenolic compound was carried out by aluminium chloride Folins and Ciocalteau reagent method .In this methodthe totalflavonoids content and phenolic content of *B. ceiba* ethanolic and aqueous barks extracts was found to be 6.272, 3.363 and 2.607, 1.607mg /100mg respectively and total phenolic and flavonoids content of *B. ceiba* ethanolic, aqueous leaves extracts was found to be 7.381, 4.590 and 3.200, 1.792 mg/100mg respectively. These studies provided information for standardization and correct identification of this plant material. These information will also be helpful to differentiate *B. ceiba* from the closely related other species. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: Pharmacognostic, Physicochemical, Qualitative, Quantitative phytochemical, *Bombax ceiba L*

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INTRODUCTION

Plant material may differ in its phytochemical content and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection and with different environmental factors surrounding the cultivation of a meticulous medicinal plant[1]. Standardization of usual products is a complex job due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible class of herbal products, proper control of starting material is greatest essential. The first step is onward ensure thequality of starting plant material authentication. Despite the advance techniques, identification of plant drugs by pharmacognostic parameters studies is more reliable. According to the WHO, the macroscopic and microscopic explanation of medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken[2]. The medicinal properties of plants are due to some chemical constituents that produce certain pharmacological action on the humans. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques[3]. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available[4]. Extensive effort have now been channelled towards screening of plants for more active and effective new drugs

to eliminate diseases which have strains of pathogenic organism that oppose the effect of drug in use today[3]. Based on the many ethno medicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition. *Bombax ceiba* (Bombacaceae), known as the silk cotton tree is a large deciduous tree found throughout India, tropical and sub-tropical regions of Asia, Africa and Australia[5]. It is an important medicinal plant of tropical and subtropical India and is well known for its traditional importance in society for centuries. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani where its potential have been highlighted for the treatment of numerous ailments like microbial infections, algesia, hepatotoxicity, hypertension, angiogenesis, HIV, fever, dysentery, catarrhal affection, ulceration of the bladder, acne, gynecological disorders, piles and urinary inflammation, infections[6,7]. Its roots and flowers are regarded as having diuretic, laxative, tonic and restorative properties, while the leaves are reported to have application in the treatment of skin eruptions. The tender bark is used as famine food, demulcent, emetic and tonic, and its aqueous extract mixed with curd to check blooddysentery[8]. A wide range of chemical constituents have been reported in *B. ceiba* i.e. shamimicin, lupeol, quercetin, hentriacontane, quercetin, rutin, anthocyanin, vicenin, fraxetin, quercetin-3-O-β-D-glucurono-pyranoside, vitexinisovitexin, kaemp-ferol3-O-β-D-glucuronopyranoside, scopolin, iso-mangiferin, mangiferin, 7-O-methyl mangiferin, esculetin,scopoletin, blumenol C glucopyranoside[7].7-hydroxycadalene,benzyl-βD-glucopyranoside, Ceibanaphthaquinone, phenylethylrutinoside,kaempferol-3-O-rutinoside, protocatechuic acid,sexangularetin-3-O-sophoroside, chlorogenic acid, quercetin-3-O-β-D-glucopyranoside, methyl chlorogenate, iso-hemigossylic acid lactone-2-methyl ether, and vanillic acid are the primary components present in plant particularly root, bark, stem and flower part [9-11]. The aim of this work was to determine the quality (types) and quantity (amount) of bioactive compounds in the leaf and bark of *B. ceiba* in Bhopal region of Madhya Pradesh.

MATERIALS AND METHODS

Materials

The Plant part (leaves and bark) of *Bombax ceiba* was collected from local area of Bhopal (M.P.).

Chemical andreagents

Sodium carbonate, acetic acid, ammonia, pyridine, potassium dehydrogenate phosphate, ferric chloride, chloroform, ethanol were obtained from Merck India Ltd. (Mumbai, India). Nitro-blue Tetra zolium, Di potassium hydrogen phosphate were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical base.

Extraction Procedure

Defatting of plant material

Powdered Plant material (leaves and bark) *Bombax ceiba* was shade dried at room temperature. The shade dried leaves and bark were coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction

80 g. of *Bombax ceiba* dried Plant material (leaves and bark) were successive extracted with various solvent (chloroform, ethyl acetate, ethanolic and aqueous) and using different drug: solvent ratios using hot continuous percolation for different time. The extracts were evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts [12] [12].

Macroscopic and microscopic Studies

The macromorphology of the barks were studied according to standard methods[13,14]. Hand section of the bark was taken, colour, odor, taste was determined. Powder was boiled with chloral hydrate to remove coloring matter and viewed under microscope after mounting it on a glass slide using glycerin covering with a cover slip .Then the powder was strained with phloroglucinol in the presence of hydrochloric acid for the lignified structure and again it was viewed under microscope as describe earlier .Further iodinewas used to locate the starch.

Physicochemical study

Determination of loss on drying

Two grams of crude powder was taken in an evaporating dish and then dried in an oven at 105°C till constant weight was obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

Determination of total ash

Two grams of dry powder was taken in a silica crucible and heated gradually increasing the heat to 500°C until it was white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without delay. Total ash value was calculated as mg g-1 of air-dried material.

Determination of water soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 min. The insoluble matter was collected on an ash less filter paper. It was washed with hot water and heated in a crucible for 15 min. Weight of insoluble matter was subtracted from the weight of total ash. The content of water soluble ash was calculated in mg g-1 of air dried material.

Determination of acid insoluble ash

Twenty five ml of hydrochloric acid (70 g/l) was added to the crucible containing total ash. It was covered with a watch-glass and heated gently for 5 min to boil. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and it was washed with hot water until the filter was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; it was dried on a hot plate and heated till constant weight was obtained. The residue was allowed to cool in desiccators for 30 minutes and then weighed without delay. Acid insoluble ash was calculated in mg/ g of air dried material[15].

Thin Layer Chromatography (TLC)

Thin layer chromatography studies of the different extracts were carried out in various solvents using Silica gel G as adsorbent and the Rf values were determined.

Qualitative phytochemical analysis of plant extract

The *Bombax ceiba* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[16,17]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates,

flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from flower of *Bombax ceiba* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso*et al*[18]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso*et al*[18]. 1 ml of 2% $AlCl_3$methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

RESULTS AND DISCUSSIONS

Macroscopic Characters

The pieces of bark of *Bombaxceiba* is are generally curved fragments, 0.5-1 cm thick, freshly collected bark is brownish internally and externally light grey in colour, but on drying they turn from grey to dark brown in colour. Bark is covered with hard, sharp, conical prickles. The eyecatching, spiny trunk of young trees becomes smoother and strongly buttressed with age. The outer surface of the bark having more roughness finely to coarsely rough with vertical and transverse cracks occasionally at places, longitudinally wrinkled and fissured. The inner surface is comparatively fine, brown in color, fracture and texture is fibrous. The bark is acrid in taste, odorless, and possesses slightly astringent flavours. Leaves are large, spreading, glabrous, leaflets lanceolate, 3-7 and margin entire.

Microscopical Characters

Powder microscopy of stem bark

Microscopy of bark Powder showed fragments of surface view of cork cells, groups of stone cells andmany free stone cells are also observed, parenchymatous cells, starch grains and prismatic crystals of calcium oxalate, phloem fibres and numerous reddish brown coloured masses Fig. 1.

The crude extracts so obtained after the hot continuous percolation extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, ethyl acetate, ethanol and water as solvents are depicted in the Table 1.

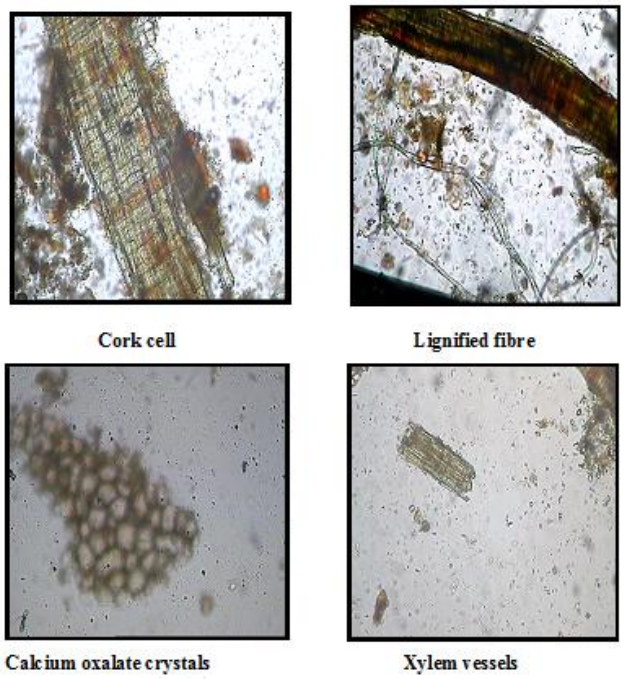

Figure 1: Powder microscopic characteristic of bark of *Bombaxceiba*

Table 1: Results of % Yield of Bombax*ceiba*

S. No.	Type of extracts	% Yield of Bombax *ceiba*	
		Bark	Leaves
1.	Chloroform	4.65	5.65
2.	Ethyl acetate	5.69	7.12
3.	Ethanol	8.98	9.45
4.	Aqueous	8.12	9.15

The physical continuousestimation of the drugs is an essential parameter to determine adulteration or inappropriate handling of drugs. The physicochemical characters of powder drug of bark of *B. ceiba* such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, and water soluble ash, loss after drying and foreign substances are given in Table2. The leaves and bark showed less moisture content; it was range from 6.5-7.5%. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. These can serve as a valuable basis of information and provide suitable standards to establish the quality of this plant material as a future prospects.An ash values are used to decide quality and purity of crude drug, it indicates presence of various impurities like, silicate, oxalate and carbonate.

Table 2: Physicochemical parameters of Bombax *ceiba bark*

S. No.	Physicochemical Parameters	% w/w
1	Total Ash	13.2
2	Acid in Soluble ash	9.7
3	Water Soluble ash	2.8
4	Water Soluble Extractive Value	5.8
5	Alcohol Extractive Value	6.7
6	Loss on drying	9.1
7	Foreign matter	1.2

The water soluble ash is used to determine the quantity of inorganic compounds present in drugs. The acid insoluble ash helps to estimate the amount of silica present in the material. The total water soluble portion of the ash is considered as water soluble ash. Less amount of these three parameters indicate that the inorganic matter and silica were less in Bombax *ceiba bark*.

The results of qualitative phytochemical analysis of the crude powder of leaves and bark parts of *Bombax ceiba* are shown in Table 3 & 4. Ethanolic and aqueous extracts of bark and leaves sample of *Bombax ceiba* showed the presence of flavonoids, phenols, tannins, carbohydrate, glycosides and proteins but in chloroform and ethyl acetate extracts all phytoconstituents was absents.

Table 3 Phytochemical evaluation of *Bombax ceiba* bark extracts

Chemical Tests	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids				
Hager's reagent	-	-	-	-
Wagner's reagent	-	-	-	-
Dragendorff's reagent	-	-	-	-
Glycosides (+Ve)				
Legal's test	-	-	+	+
Phenols/Tannins				
Ferric chloride	-	-	+	+
Flavonoids				
Lead acetate test	-	-	+	+
Alkaline reagent test	-	-	+	+
Saponins				
Foam test	-	-	-	+
Fixed oil/Fats				
Spot	-	-	-	-
Saponification	-	-	-	-
Carbohydrates				
Molish test	-	-	+	+
Fehling's solution test	-	-	+	+
Amino acids				
Xantoprotein Test	-	-	-	-
Protein				
Biuret test	-	-	+	-

Table 4: Phytochemical evaluation of *Bombax ceiba* leaves extracts

Chemical Tests	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids				
Hager's reagent	-	-	-	-
Wagner's reagent	-	-	-	-
Dragendorff's reagent	-	-	-	-
Glycosides (+Ve)				
Legal's test	-	-	+	+
Phenols/Tannins				
Ferric chloride	-	-	+	+
Flavonoids				
Lead acetate test	-	-	+	+
Alkaline reagent test	-	-	+	+
Saponins				
Foam test	-	-	-	+
Fixed oil/Fats				
Spot	-	-	-	-
Saponification	-	-	-	-
Carbohydrates				
Molish test	-	-	+	+
Fehling's solution test	-	-	+	+
Amino acids				
Xantoprotein Test	-	-	-	-
Protein				
Biuret test	-	-	+	-

(+) Indicates 'Presence'; (-) Indicates 'Absence'

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of ethanolic and aqueous extract of *Bombax ceiba* leaves and bark showed the content values of 7.381, 4.590 and 6.272, 3.363 respectively. But chloroform and ethyl acetate extracts of *Bombax ceiba* leaves and bark have no phenolic content. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of ethanolic and aqueous extracts of leaves and bark of *Bombax ceiba* showed the content values of 3.200, 1.792 and 2.607, 1.607 respectively. The above results showed that aqueous extract contain less phenolic and flavonoids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents. Results are provided in (Table 5, 6 and Fig. 2, 3).

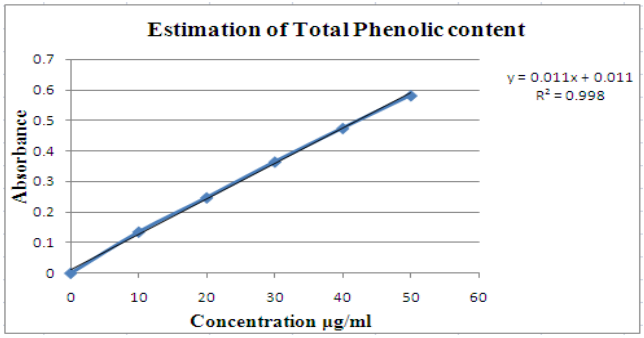

Figure 2: Graph of estimation of total phenolic content

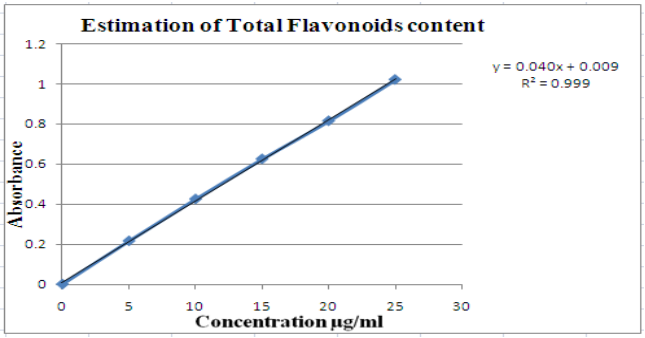

Figure 3: Graph of estimation of total flavonoids content

Table 5: Estimation of TPC and TFC of *Bombax ceiba* leaves extracts

Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
Chloroform	-	-
Ethyl Acetate	-	-
Ethanol	7.381	3.200
Aqueous	4.590	1.792

Table 6: Estimation of TPC and TFC of *Bombax ceiba* bark extracts

Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
Chloroform	-	-
Ethyl Acetate	-	-
Ethanol	6.272	2.607
Aqueous	3.363	1.607

CONCLUSION

Following present investigation it can be concluded that the standardization and preliminary phytochemical investigation study of *Bombax ceiba* leaves and bark yielded a set of standards that can serve as an essentialbasis of evidence to determine the identity and to determine the quality and purity of the plant material as per its future perspectives. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of leaf and bark to establish as the drug. It would also help in distinguishing the plant material of *Bombax ceiba* from its related species. The phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemicals have been found to possess a wide range of activities.

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CONFLICT OF INTEREST

Authors declare, there is no conflict of interest.

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