



PHYTOCHEMICAL ANALYSIS AND NUTRITIVE VALUE OF *BOMBAX CEIBA* LINN. (PETALS)

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Abstract

Bombax ceiba L. is wild, non-cultivated and has become one of the traditionally important trees in India. The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of plant *Bombax ceiba* L. in some solvents (petroleum ether (P.E) chloroform (C.H), ethyl acetate (E.T) ethanol (E.L), and water (W.R)). Flower collected from haridwar region, Uttarakhand. Qualitative phytochemicals such as alkaloids, flavanoids, glycosides, carbohydrates, terpenoids, proteins and tannins were detected when examining the Flower Petals of *Bombax ceiba* L. Quantitative phytochemical i.e., alkaloids, glycosides saponins, flavonoids and tannin were found higher concentration in polar solvent ethanol extract. While extract of non polar solvent, petroleum ether has lower in concentration. The obtained data from *Bombax ceiba* L. (petals) confirmed its wide application for therapeutic purpose in alternative therapy.

Key words: *Bombax ceiba* L. (petals), Qualitative phytochemicals, nutritive value, secondary metabolites.

Introduction

Phytochemicals are important components present in a plant material ('phyto' is from the Greek word meaning plant) that exert protective or disease-preventing effects (Surh, 2003). Phytochemical is a term meaning plant chemical referring to a wide variety of compounds that present naturally in plants. The term bioactive has also broad meaning defined as bioactive compounds, those have ability to interact with other components of living tissues representing a wide range of biological effects (Huang *et al.*, 2016). Plants are major source of secondary metabolites which are formed as products of primary metabolism and produced for defense against predators (Unuofin *et al.*, 2017). Phytochemicals have been associated with protection from and treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes and other medical conditions. Phytochemicals have been divided into six categories on the basis of their chemical structures and characteristics. These categories include lipids, phenolics, carbohydrate, terpenoids and alkaloids, and other nitrogen-containing compounds (Campos-Vega and Oomah, 2013). Within

each category, further division based on biosynthetic origin gives rise to further subcategories. Examples of such metabolites are tannins, alkaloids and flavonoids; they are known to be the brain behind the healing potentials of plants (Bhandary, 2012). Traditional medicine is the oldest method of curing diseases. Many plants have been used in different parts of the world to treat human diseases and infections (Pranoothi *et al.*, 2014). Plants are used medicinally in all over the world and are a source of many potential and powerful drugs. Traditional medicine have been using plant extracts to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). More than 5,000 phytochemicals have been identified, and many are still unknown (Liu, 2013). Some phytochemicals (e.g., β -carotene) have been linked to obesity prevention, although the evidence is not as strong (Diep, 2015). Phytochemical structure, which influences solubility, and cellular localization of phytochemicals are two critical factors influencing their retention during processing. The relatively polar phytochemicals including various classes of phenolics (hydroxybenzoic and hydroxycinnamic acids) and flavonoids (primarily anthocyanins, flavonols, flavones, procyanidins) are readily leached into water during

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blanching and syrups/brines during canning. In contrast carotenoids are well retained during blanching and canning due to their non-polar nature and resistance to leaching, although losses can occur due to thermal degradation and oxidation. Flavonoids are contained within hydroxyl groups membrane complexes. Flavonoids are mostly isolated from plant extracts. Phenolic compounds include phenolic acids, polyphenols (tannins) and flavonoids (Quy *et al.*, 2014). These compounds protect plants, fruits, and vegetables from oxidative damage and have been used as antioxidants by humans. *Bombax ceiba* L. (petals), which is commonly known as “Simal”, is one of the oldest spices of genus *Bombax* L. Nowadays, By tradition, flowers have been used as a cure for sexual impotency, hemorrhoids, swelling, and boils, and barks for cough, indigestion and stomach ache (Chopra *et al.*, 1956). It has been widely used in both Chinese and Indian traditional medicine for the treatment of diarrhea, fever, chronic inflammation, and catarrhal affection (Wu *et al.*, 2008). So it possesses medicinal properties and is used in many formulations. The aqueous extract of *Bombax ceiba* L. flowers exhibited a cardioprotective effect and the methanolic extract exhibited antioxidant activity (Patel *et al.*, 2011). Some different parts of the *Bombax ceiba* is used for medical purposes However, many species include phytochemicals alkaloids, flavonoids, fatty acids, which are responsible for anti-oxidant, anti-inflammatory, anti-allergic, anti-bacterial activities (Said *et al.*, 2011).

The aim of the present research is to explore the qualitative phytochemical and nutritive analysis of *Bombax ceiba* L. (petals).

Materials and Methods

Chemicals and instruments

Solvents and chemicals used were purchased from Merck and Sigma–Aldrich. These included Folin–Ciocalteu reagent, anhydrous sodium carbonate, aluminium trichloride (AlCl₃), sodium nitrite, sodium chloride potassium acetate, ferric chloride, ascorbic acid, n-butanol, diethyl ether, ammonia solution, acetone, ethanol, hydrochloric acid, sodium hydroxide, phosphate buffer, potassium ferricyanide, ammonium molybdate, sodium phosphate, trichloroacetic acid, glacial acetic acid and sodium nitroprusside. All the chemicals used in this study were of analytical grade.

Collection, identification and authentication of flower

B. Ceiba was collected from campus of Gurukul Kangri Vishwavidyalaya, Haridwar (daytime air temperature, 12-17.2°C) of Uttarakhand of India in the

month of January 2017 and authenticated from Botanical survey of India (BSI) Dehradun (Voucher specimen number 117964 08/2017). Flower petals were separated and dried for 15–20 days under shade until petals seem ready for grinding and stored at room temperature, were subjected to grinding in a laboratory grinder and stored at 4°C (Shukla *et al.*, 2019).

Preparation of crude flower extract

Dry powdered material of petals (200 g) were packed into a Soxhlet apparatus and extracted with 800 ml of each solvent successively in increasing order of polarity. The extracts were filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure at 40°C. The extracts were dried, weighed and stored at 4°C storage vials for experimental use (Kaur *et al.*, 2018).

$$\text{Yield \%} = \frac{\text{Weight of extract}}{\text{weight of dried plant material}} \times 100$$

Proximate analysis

Ash Content:

Five gram of each leaf sample was weighed in a silica crucible and heated in muffle furnace for about 5-6 hours at 550°C. It was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash become white or grayish white). The weight of ash was measured.

Moisture Content:

Two gram of each sample was taken in a flat-bottomed dish and kept overnight in an air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Crude fat Content:

Two gram of dry of each sample was extracted with petroleum ether at 60-80°C in a Soxhlet apparatus for about 6-8 hours. After boiling with petrol, the residual petrol was filtered using Whatman no: 40 filter paper and the filtrate were evaporated in a pre-weighed beaker. Increase in weight of the beaker was measured as the weight of crude fat.

Table 1: Yield & colour of extracts of *Bombax ceiba*. (petals).

Plant extract	Bark extraction	
	yield %	Colour
Petroleum ether	1.32	Light yellow
Chloroform	0.892	yellow
Ethyl acetate	9.122	yellow
Ethanol	4.91	yellow
Water	22.194	Brown

Crude fibre Content:

Two gram of moisture and fat-free material of each sample was treated with 200ml of 1.25% H₂SO₄. After filtration and washing, the residue was treated with 1.25% NaOH. It was filtered, washed with hot water and then 1% HNO₃ and again with hot water. The washed residue was dried in an oven at 130°C to constant weight and cooled in a dessicator. The residue was scraped into a pre-weighed porcelain crucible, weighed, ashed at 550°C for two hours, cooled in a dessicator and reweighed.

Crude protein Content:

The crude protein was determined using micro Kjeldahl method. The total protein was calculated multiplying the evaluated nitrogen by 6.25.

Carbohydrate Content:

Percentage of available carbohydrate was calculated using the formula,

$$\% \text{ of carbohydrate} = 100 - [\% \text{ of ash} + \% \text{ of fat} + \% \text{ of protein} + \% \text{ of fiber}]$$

Nutritive value (energy) analysis:

Nutritive value of each plant sample was determined by multiplying the values obtained for protein, fat and available carbohydrate by (4:9:4) respectively and adding up the values.

Estimation of Nutritive Value (Energy) or Calorific Value:

$$\text{Nutritive Value} = 4 \% \text{ of protein} + 9 \% \text{ of fat} + 4 \% \text{ of carbohydrate}$$

Table 2: Qualitative phytochemical screening of *Bombax ceiba* (petals).

Phytoconstituents and Test performed		Extracts					
		PE	CH	ET	EL	WR	
Alkaloids	Mayer's Test	-	-	-	-	-	
	Wagner's Test	-	-	-	-	-	
	Hager's Test	-	-	-	-	-	
	Tannic acid Test	-	-	-	-	-	
Carbohydrates	Molisch's Test	+	+	+	+	+	
	Fehling's Test	-	-	+	+	-	
	Benedict's Test	-	+	+	+	-	
	Selivanoff's Test	-	-	+	-	-	
Glycosides	Anthraquinone glycosides	Borntragger's Test	-	-	-	-	-
		Test for hydroxy-anthraquinones	-	-	-	-	-
	Cardiac glycosides	Keller-Killiani Test	-	-	+	+	-
		Legal's Test	+	+	+	+	-
		Baljet's Test	-	-	+	+	-
	Saponins glycosides	Froth formation Test	-	-	-	+	-
Flavonol glycosides	Mg and HCl reduction	+	+	+	+	-	
Inulin							
Protein	Heat Test	-	+	-	-	-	
	Biuret Test	+	+	-	-	-	
	Xanthoproteic Test	-	-	-	+	-	
Amino acids	Ninhydrin Test	-	-	-	-	-	
Steroids and Triterpenoids	Salkowski Test	-	+	+	+	-	
Fixed oils and Fats	Spot Test	+	-	-	-	-	
	Saponification Test	+	+	-	-	-	
Flavonoids	Shinoda Test	-	-	-	+	-	
	Alkaline reagent	-	-	+	+	-	
	Zinc hydrochloride Test	-	-	-	-	-	
Phenolic compounds and Tannins	Lead Acetate Test	-	-	-	-	-	
	Ferric chloride Test	-	-	+	+	-	
	Test for Catechin	-	+	-	-	-	
	Test for chlorogenic acid	-	+	-	-	-	
Gums and Mucilage		-	-	-	-	-	
Taphthoquinone	Juglon Test	-	-	-	-	-	
	Dam-Karrer Test	-	-	-	-	-	

Qualitative phytochemicals

The Bioactive compounds were analysed by the qualitative tests for the solvent extracts. It was screened for alkaloids, flavonoids, cardiac glycosides, carbohydrates, terpenoids, protein and tannins by using standard procedures followed by (Mahendra *et al.*, 2017).

Detection of Alkaloids

Extracts were dissolved in dilute HCl and then filtered. Different test have done for screening of alkaloid present in *Bombax ceiba* L. (petals).

Mayer's test: Filtrates were treated with Mayer's reagent. *Yellow color* precipitate indicates presence of alkaloid.

Dragendroff's test: Filtrates were treated with Dragendroff's reagent. *Red* precipitate indicates presence of alkaloids.

Hager's test: Filtrates were treated with Hager's reagent. *Yellow* precipitate indicates presence of alkaloids.

Detection of Flavanoids

Alkaline Reagent test: Extracts were treated with few drops of NaOH solution. Formation of intense Yellow color which becomes colourless on addition of dilute acid (HCl or H₂SO₄) indicates the presence of Flavanoids.

Lead Acetate test: Extracts were treated with few drops of Lead Acetate solution. Formation of intense Yellow coloured precipitates indicates the presence of Flavanoids.

Detection of Glycosides

Extracts were hydrolyzed with dilute HCl and filtered.

Modified Bortrager's test: Extracts were treated with 5 % Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was than cooled and extracted with equal amount of benzene. The upper layer was separated and treated with Ammonia solution. Formation of Rose Pink colour in the Ammonical layer

indicates the presence of glycosides. (Anthranol glycosides).

Legal test: Extracts were treated with sodium nitroprusside in Pyridine and NaOH. Formation of Pink to blood Red colour indicates the presence of glycosides. (Cardiac glycosides).

Keller killiani test: Extracts mixed with chloroform and evaporate to dryness. Add 0.4 ml glacial acetic acid containing trace amount of ferric chloride. Transfer it to test tube and add carefully 0.5 ml of concentrated H₂SO₄ by the side of the test tube. Acetic acid layer shows blue colour indicates the presence of glycosides.

Inulin

Test solution as treated with a mixture of á-naphthol and sulphuric acid, brownish red colour is formed which indicate the presence of inulin.

Detection of Carbohydrates

100 mg extracts were dissolved in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch Test: Filtrates were treated with a drop of alcoholic naphthol solution in a test tube. Formation of Violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently in water bath. An orange red precipitate indicates the presence of reducing sugar.

Barfoed's Test: To 1 ml filtrate 1 ml of Barfoed's reagent is added and heated on a boiling water bath for 2 minutes. A red precipitate indicates the presence of sugar.

Fehling's Test: 1ml filtrate is boiled water bath with 1 ml of each Fehling solution A and B. A red precipitate indicates the presence of sugar.

Detection of Tannins

Ferric Chloride Test: To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for Gallic tannins and green for catecholic tannins.

Detection of Terpenoids

Salvoskii Test: 5 ml of each extract was mixed with chloroform 3 ml of concentrated H₂SO₄ was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids.

Detection of Protein test and amino acid test

Millon's Test: To 2 ml of 5 ml of extract, few drops of Millon's reagent are added. A white precipitate shows

Table 3: Proximate analysis results of *Bombax ceiba* petals.

Parameters	Result %
Moisture	7.4
Crude protein	3.79
Crude Fat	15.75
Ash	7.02
Crude fiber	16.9
Total carbohydrate	66.04

Table 4: Nutritive value of *Bombax ceiba* (petals).

Plant	Part	Nutritive value result (Kcal/100gm)
<i>Bombax ceiba</i> L.	Bark	353.47

the presence of protein.

Biuret Test: An aliquot of 2 ml of extract with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) is added followed by excess of KOH pellets. Pink colour in the ethanol layer indicates the presence of protein

Ninhydrin Test: 2 drops of Ninhydrin solution (10 mg of ninhydrin 200 ml of acetone) are added to 2 ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acid.

Results and Discussion

The results for extraction process of *Bombax ceiba* L. (petals) from all solvents showed in Table 1. Base on the results in Table 1, polar solvent ethyl acetate and water have higher in the extract and % rendement compare to non polar solvent (petroleum ether). Qualitative phytochemical characteristics of *Bombax ceiba* L. (petals) are summarize in Table 2. The bioactive component *i.e.* saponins, flavonoids and tannin were found in different solvents. The higher concentration of flavonoid, saponin and tannin were resulted from polar solvent ethanol. While non polar solvent and water extract has lower in concentration. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari *et al.*, 2011). Flavonoids are well documented for the biological effects including antimicrobial and anticancer (Demetrio *et al.*, 2017). They have been found in vitro to be effective antimicrobial and anticancer compounds against a wide array of microorganism and cancer cell (Harbone, 1998). Bioactive constituent have been reported to be responsible for medical herbs in Chinese and Japanese (Njoku and Obi, 2009). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the petals of the flower studied. The presence of some of these compounds have also been confirmed to have antioxidant and antimicrobial activity (Kavit *et al.*, 2012). Proximate analysis results reveals that petals of *Bombax ceiba* are good source of fat, fibre, moisture and carbohydrates. Nutritive value of flower is 353.47. Hence it could be concluded that the petals of extracts of *Bombax ceiba* L. (petals) could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Conclusion

In the present study ethanol extract showed the presence of flavonoids, terpenoids, tannins, saponins and

other phytochemicals by qualitative method. Water extract showed less amount of phytochemicals in comparison to all other extract of *Bombax ceiba* L. (petals). Petals are good source of fat, fibre and carbohydrate which reflects a good nutritive value. This study also leads to the further research in the way of isolation and identification of the active compound from the selected *Bombax ceiba* L. (petals) using chromatographic and spectroscopic techniques.

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