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NUTRITIONAL IMPORTANCE AND PHYTOCHEMICAL INVESTIGATION OF SEPALS OF *BOMBAX CEIBA* LINN.

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Abstract

The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These non-nutritive components are called phytochemicals. The qualitative analysis as well as quantification of phytochemicals of a medicinal plant is regarded as vital step in any kind of medicinal plant research. *Bombax ceiba* L. is one of the important medicinal trees in India. The present assessment investigates the phytochemical and proximate examination of the major bioactive constituents of sepals of *Bombax ceiba* L. in certain solvents petroleum ether (P.E) chloroform (C.H), ethyl acetate (E.T) ethanol (E.L) and water (W.R) Flower assembled from Haridwar, Uttarakhand. Subjective phytochemicals comprising of alkaloids, flavonoids, glycosides, saponins, terpenoids, proteins, and tannins have been distinguished when reviewing the Flower Sepals of *Bombax ceiba* L. Quantitative phytochemical *i.e.*, alkaloids, glycosides saponins, flavonoids, and tannin were discovered higher focus in polar solvent ethanol. While they concentrate on non-polar dissolvable, petroleum ether was found less active. The results obtained from *Bombax ceiba* L. (sepals) affirmed its wide application for remedial purposes in healthcare treatment.The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.

Key words: *Bombax ceiba,* pytochemicals, proximate analysis and nutritive value.

Introduction

Phytochemicals are naturally occurring biochemicals in plants which are responsible for colour, flavour, smell and texture. Preliminary phytochemical screening of medicinal plants is a useful method for qualitatively determination of different metabolite in crude sample. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsycohotic drugs (Thamizharasan *et al*., 2015). There are hundreds of medicinal plants that have a long history of curative properties against various diseases and aliments. Plants have limitless ability to synthesizearomatic substances, mostly phenols or theiroxygen-substituted derivatives (Santhi *et al*., 2016). Most of the natural products aresecondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defensemechanisms against predation bymicroorganisms, insects and herbivores (Kokate *et al*., 2004). Today thereis growing interest in chemical compositionof plant based medicines. Several bioactiveconstituents have been isolated and studied for pharmacological activities. During thelast two decades, the pharmaceuticalindustry has made massive investment inpharmacological and chemical researches allover the world in an effort to discover muchmore potent drugs, rather, a few new drugs. Plants have successfully passed the tests ofcommercial screenings. To treat chronic disease and production of drugs with precise activity, relationship between the phytochemical compounds and the bioactivity of medicinal plant is very much important (Sathya *et al*., 2013). Evaluation of crude drug is important for discovery of new sources of low price homoeopathic ingredients which then lead to the synthesis of modern chemical compound for treating severe diseases (Mir *et al*., 2013). Phytochemical screening gives us hints and evidences to the discovery of new valuable Drugs (Chithra *et al*., 2013).

Sepal of *B. Ceiba* has not been the subject of any phytochemical or proximate studies before than that to our knowledge. In this study, we are interested in studying for the first time the chemical composition of the different extractsand nutritive value of sepal. It should be noted that to date, no studies on the chemical composition and nutritive value of sepals offlower of *Bombax ceiba* species has been made.

Material and Method

Chemicals and instruments

Solvents and chemicals used were purchased from Merck and Sigma-Aldrich. These included Folin- Ciocalteu reagent, anhydrous sodium carbonate, aluminium trichloride ($AlCl_3$), 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 Gurukulkangri 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 sepals were isolated and dried for 10-15 days under shade until sepals appear to be prepared for crushing and put

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away at room temperature, were exposed to granulating in a research center processor and put away at 4°C (Kaur *et al*., 2019).

Preparation of crude flower extract

Dry powdered material of sepals (200 g) were packed into a Soxhlet apparatus and extracted with 800ml 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 4ºC storage vials for experimental use (Kaur *et al*., 2018).

Yield% = Weight of extract / weight of dried plant material×100

Proximate analysis

Proximate analysis of sepals of *Bombax ceiba* was analysed by the method followed by (Shukla *et al*., 2015).

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 550ºC. 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 100-110ºC 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 60-80ºC 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 preweighed beaker. Increase in weight of the beaker was measured as the weight of crude fat.

Crude fibre Content: Two gram of moisture and fat-free material of each sample was treated with 200ml of 1.25% H_2SO_4. After filtration and washing, the residue was treated with 1.25% NaOH. It was filtered, washed with hot water and then 1% HNO_3 and again with hot water. The washed residue was dried in an oven at 130ºC to constant weight and cooled in a dessicator. The residue was scraped into a pre-weighed porcelain crucible, weighed, ashed at 550ºC 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,

% of carbohydrate = 100 - (% of ash+ % of fat+ % of protein + % 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:

Nutritive Value = 4 % of protein + 9 % of fat + 4 % of carbohydrate

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 (Shukla *et al*., 2014).

Detection of Alkaloids

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

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 H_2SO_4) 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 Borntrager'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 chloroformand 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 H_2SO_4 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 α-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.

Bendict's Test: Filtrates were treated with Bendict'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: 1 ml filtrate is boiled water bath with I 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

Salwoskii Test: 5 ml of each extract was mixed with chloroform 3 ml of concentrated H_2SO_4 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 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.

Result and Discussion

The results for extraction process of *Bombax ceiba L.* (sepals) 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, chloroform). Proximate analysis results reveals that sepals of *Bombax ceiba* are good source of fat, fibre, moisture and carbohydrates, on the other hand sepals contain less amount of protein (Table 2). Nutritive value of flower is 373.78 Kcal/100gm (Table 3). Qualitative phytochemical characteristics of *Bombax ceiba L.* (sepals) are summarize in Table 4. The bioactive component i.e., saponins, flavonoids and tannin were found in different solvents. Flavonoid, saponin and tannin were present in polar solvent ethanol. While non polar solvent and water extract was found less active. 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. They have been found in vitro to be effective antimicrobial and anticancer compounds against a wide array of microorganism and cancer cell. 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 sepals 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). Hence it could be concluded that the sepals of extracts of *Bombax ceiba L.* (sepals) could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Conclusion

In the present investigation, ethyl acetate extract indicated the presence of different phytochemicals like flavonoids, terpenoids, tannins, saponins etc. Water extract indicated less measure of phytochemicals in contrast with all other concentrate *of Bombax ceiba L*. (sepals). Sepalsare good source of fat, fiber, and carbohydrates which mirrors a decent nutritive value. This investigation likewise prompts further research in the method for segregation and ID of the dynamic compound from the chose *Bombax ceiba L.* (sepals) utilizing chromatographic and spectroscopic procedures.

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Table 1: Yield and colour of extracts of *Bombax ceiba L. (*sepals*)*

Plant extract	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

Table 2: Proximate analysis results of *Bombax ceiba L.* (sepals)

Parameters	Result %
Moisture	5.90
Crude protein	4.31
Crude Fat	17.86
Ash	7.18
Crude fiber	15.8
Total carbohydrate	64.74

Table 3: Nutritive value of *Bombax ceiba L.* (sepals)

Plant	Part	Nutritive value result (Kcal/100gm)
Bombax ceiba L.	Bark	373.78

Table 4: Qualitative phytochemical screening of *Bombax ceiba* L. (sepals*)*

Phytoconstituents and Test performed			Extracts				
			P.E	C.H	E.T	E.L	W.R
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 and Amino acids	Millon's Test		-	-	-	+	-
	Biuret Test		+	+	-	-	-
	Ninhydrin Test		-	-	-	-	-
Steroids and Triterpenoids	Salkowski Test		-	+	+	-	-
Fixed oils and Fats	Spot Test		+	-	-	-	-
	Saponification Test		+	+	-	+	-
Flavonoid test	Alkaline reagent		-	-	+	+	-
	Lead Acetate Test		-	-	-	+	+
Phenolic compounds and Tannins	Ferric chloride Test		-	-	-	+	+