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Phytochemical analysis and nutritive value of *Bombax ceiba Linn*. (Anther filament)

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ABSTRACT
Bombax ceiba L. is wild, commonly known trees in India. The present study evaluation explores quantitative assessment of nutritional importance and qualitative assessment the major bioactive constituents of flower anther of Bombax ceiba Linn. in specific solvents petroleum ether (P.E) chloroform (C.H), ethyl acetate (E.T) ethanol (E.L), and water (W.R). Flower gathered from Haridwar region, Uttarakhand, subjective phytochemicals involving alkaloids, flavonoids, glycosides, terpenoids, proteins, and tannins have been recognized when looking into the Flower anther filament of Bombax ceiba.Qualitative phytochemical ie., alkaloids, glycosides, flavonoids and tannin were found higher concentration in polar dissolvable ethanol discrete, while non-polar dissolvable, petroleum ether was found less active in screening of such phytochemicals. The data obtained from Bombax ceiba L. (anther filament) attested its wide application for healing purposes in health treatment.
Keywords: *Bombax ceiba, pytochemicals, nutritive value, secondary metabolites.*

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INTRODUCTION

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 [1]. Plants are used medicinally in all over the world and are a source of many potential and powerful drugs. Traditional medicines have been using plant extracts to provide health coverage for over 80% of the world's population, especially in the developing world [2]. Phytochemicals are important components present in a plant material, have exert protective or disease preventing effects [3]. Phytochemical is a term meaning plant chemical referring to a wide variety of compounds that present naturally in plants and responsible for biological activities. 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 [4]. Plants are major source of secondary metabolites which are formed as products of primary metabolism and produced for defense against predators [5]. 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 [6]. Within each category, further division based on biosynthetic origin gives rise to further subcategories. Examples of such metabolites are tannins, alkaloids and flavonoids [7]. More than 5,000 phytochemicals have been identified, and many are still known for their biological effect [8]. Some phytochemicals like β-carotene has been linked to obesity prevention [9]. Flavonoids are mostly isolated from plant extracts. Phenolic compounds include phenolic acids, polyphenols (tannins) and flavonoids [10]. These compounds protect plants, fruits, and vegetables from oxidative damage and have been used as antioxidants by humans.

Plant *Bombax ceiba Linn* (flower - a.f), which is commonly known as "Simal", is one of the oldest spices of genus *Bombax* Linn. Nowadays, different parts of this *Bombax ceiba* have been used as a cure

for hemorrhoids, swelling, and boils, sexual impotency, cough and stomach ache [11]. It has been widely used in both Chinese and Indian traditional medicine for the treatment of diarrhea, fever, chronic inflammation, and catarrhal affection (12). So it possesses medicinal properties and is used in many formulations. The aqueous extract of *Bombaxceiba* flowers exhibited a cardioprotective effect and the methanolic extract exhibited antioxidant activity [13]. 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 antioxidant, anti-inflammatory, anti-allergic, antibecterial activities [14]. The point of the present research is to investigate the subjective phytochemical and nutritive value of *Bombax ceiba L.*(anther filament).

MATERIAL AND METHODS

Chemicals and instruments

Solvents and chemicals used were purchased from Merck and Sigma–Aldrich. These included Folin–Ciocalteu reagent, anhydrous sodium carbonate, aluminiumtrichloride, 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.

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 anther filaments were isolated and dried for 15–20 days under shade until petals appear to be prepared for crushing and put away at room temperature, were exposed to granulating in a research center processor and store at 4 °C (15).

Preparation of crude flower extract

Dry powdered material of anther filament (200g) were packed into a Soxhlet apparatus and extracted with 800mlof each solvent successively in increasing order of polarity. The extracts were filtered by using Whatmanpaper No. 1, and the filtrate was concentrated under rotary vaccum at 40°C.The extracts were dried, weighed and stored at 4ºC storage vials for experimental use (16).

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

Proximate analysis:

Ash Content:

Five gram of each leaf raw sample was weighed in a silica crucible and heated in muffle furnace for about 4-5 hours at 550ºC. It was heated again in the furnace for half an hour, cooled and weighed. This procedure was repeated 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 the 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 petroleum ether, the residual petroleum ether 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 fat-free and moisture free material of each sample was treated with 200ml of 1.25% H_2SO_4. After filtration the residue was washed 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 desiccators. The residue was scraped into a pre-weighed porcelain crucible, weighed, ashed at 550ºC for two hours, cooled in a desiccators and reweighed.

Crude protein Content:

The crude protein was determined using Kjeldahl method.

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 with the help of the values obtained from crude protein, fat and 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 (17).

Detection of Alkaloids

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

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: Plants 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: Few drops of Lead Acetate solution was added to plant extracts. 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: Plant 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. 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 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 the presence of carbohydrates.

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

Bendict's Test: Filtrates were treated with Bendict's reagent and heated gently in water bath. 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.*(anther filament) 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).Proximate analysis results reveals that petals of *Bombax ceiba L.* are good source of fat, fibre, moisture and carbohydrates (Table 2). Nutritive value of flower is 353.47 (Table 3).

Table 1. Yield &colour of extracts of *Bombax ceiba L. (*anther filament*).*

Plant extract	Extraction	
	yield %	Colour
Petroleum ether	1.07	Light yellow
Chloroform	0.816	yellow
Ethyl acetate	4.33	yellow
Ethanol	4.06	yellow
Water	7.90	Brown

Table 2. Proximate analysis results of *Bombax ceiba L.* Anther filament

Parameters	Result %
Moisture	5.6
Crude protein	3.67
Crude Fat	13.08
Ash	9.36
Crude fiber	13.9
Total carbohydrate	68.25

Table 3. Nutritive value of Bombax ceiba (anther filament)

Plant	Part	Nutritive value result (Kcal/100gm)
Bombax ceiba L.	**Flower (A.F)**	349.92

Table 4. Qualitative phytochemical screening of *Bombax ceiba L.*(anther filament*).*

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			+	+	+	-	-
	Biuret Test		+	+	+	-	-
Amino acids	Ninhydrin Test		-	-	-	-	-
Terpenoids	Salkowski Test		-	-	+	+	-
	Alkaline reagent		-	-	-	+	-
	Zinc hydrochloride Test		-	-	-	-	-
Tannins	Lead Acetate Test		-	-	+	+	-
	Ferric chloride Test		-	-	+	+	-

Qualitative phytochemical characteristics of *Bombax ceiba L.*(anther filament) are summarize in Table 4. The bioactive component ie., flavonoids and tannin were found in different solvents. The higher concentration of flavonoid and tannin were resulted from polar solvent ethanol. While non polar solvent and water extract has lower in concentration [18]. Flavonoids are well documented for the biological effects including antimicrobial and anticancer (19). Bioactive constituent have been reported to be responsible for medical herbs in Chinese and Japanese [20]. 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 (21).Hence it could be concluded that the petals of extracts of *Bombax ceiba L.* (anther filament) could be a source for the industrial manufacture of drugs useful in the different health ailments.

CONCLUSION

In the present reveals that *Bombax ceiba* anther filaments are good source of fat, fibre and carbohydrate which reflects a good nutritive value. Qualitative phytochemical investigation shows that ethanol extract indicated the presence of flavonoids, terpenoids, tannins, saponins and different phytochemicals by subjective technique. Water extract indicated less quality of phytochemicals in contrast with all other concentrate of *Bombax ceiba L.* (anther filament). 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.*(anther)using chromatographic and spectroscopic techniques.

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