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Preliminary Phytochemical Screening of Bombax Ceiba Fruit

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Abstract: ***Bombax ceiba , family Bombacaceae is a large, beautiful deciduous tree found throughout India.Iit has stimulant, astringement, haemostatic aphrodisiac, diuretic, antidiarrheal, cardiotonic, emetic, demulcent, antidysenteric, al ternative and antipyretic properties. Various parts of the plant has been found to be effective against various ailments. More over, the fruit of the plant also possess a number of medicinal properties. However there is a lack of data corresponding to the standardization and phytochemical profile of the fruit of the plant. Therefore, some parameters related to physico-chemical properties like extractive values, ash values, loss on drying have been evaluated and were analysed as per WHO guidelines. Preliminary phytochemical screening was performed using standard chemical reagents. The preliminary phytochemical screening of the alcoholic extract validated the presence of carbohydrates, glycosides, alkaloids, sterols, phenolics and tannins, saponins, flavonoids and amino acids. The presence of different phytochemical constituents were confirmed by performing TLC which revealed the presence of various flavonoids along with steroids and amino acids. Along with various parts,the fruit of the plant has been found to be rich source of antioxidants specially flavonoids.***

Keywords: Bombax ceiba Linn., Flavonoids, Physicochemical analysis, Phyto- chemical analysis, TLC.

I. INTRODUCTION

Plant-based drugs have been used worldwide in traditional medicines for treatment of various diseases. World plant biodiversity is the largest source of herbal medicine and still about 60-80 % world population relies on plant-based medicines, which are being used since the ancient ages as traditional medicine. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world. It is now clear that the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on the human body. These natural compounds also formed the base of modern drugs as we are using today [1].

Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. Bombacaceae is a small family of the order Malvales and contains about 28 genera and 200 species. Members of this family are not only showy ornamentals but they possess significant economic and commercial reputation as well. In addition, the various parts of B. ceiba have been reported for hypotensive and hypoglycemic, anti angiogenic , analgesic , antiulcer, antioxidant , hepatoprotective and antimicrobial activities. Also it was used for the treatment of sexual debility, bleeding wounds and vaginal infections. Since there is no scientific report for anti oxidative potential of B. ceiba fruit extract, the present study was an attempt to evaluate the its antioxidative potential.(Saleem et al,1999).The physicochemical characteristics, phytochemical constituents, thin layer chromatography (TLC) analysis of fruits of *Bombax species* extracted with hexane, chloroform, Methanol, ethanol and water and also to perform qualitative and quantitative analysis of some secondary metabolites and minerals present in this plant [2],[3].

II. MATERIALS AND METHODS

A. Collection of Plant Materials

The fruit of Bombax ceiba was collected from Institute of Applied Medicines & Research (IAMR, Duhai, UP) area in the month of March' 2019. The plant materials were taxonomically identified and authenticated. A voucher specimen was deposited having the specimen Ref. No. X

B. Processing of Plant Materials

The plant Materials was cleaned and shade dried until all the water molecules evaporated and the dried plant materials (fruit) was taken and grinded into coarse powder. The powdered samples were stored in a clean glassware container until needed for analysis with proper labeling.

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C. Preliminary Physicochemical Characteristics

Air dried fruits were used for quantitative determination of proximate analysis e.g., loss on drying, total ash, acid insoluble ash, alcohol soluble extractive values. These physicochemical studies were done according to standard procedure of Indian Pharmacopoeia and WHO guidelines [4].

D. Qualitative Phytochemical Analysis

Plant extracts were dissolved in five different solvents, Hexane, Methanol, chloroform, ethanol and water. So five different extracts were prepared for further analysis. The extracts were tested for the presence of bioactive components by using following standard methods [5]-[7].

E. Phytochemical Screening

All the types of extracts were tested parallel for the presence of various phytochemicals, polar or non-polar compounds.

F. Test for Alkaloids (Wagner's test)

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of reddish brown precipitate (or coloration).

G. Test for Carbohydrates (Molisch's test)

Few drops of Molisch's reagent were added to 2 ml portion of the various extracts. This was followed by addition of 2 ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet color at the interphase of the two layers was a positive test.

H. Test for Cardiac Glycosides (Keller Kelliani's test)

5 ml of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully under layed with 1 ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

I. Test for Phenols (Ferric Chloride Test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

J. Test for Phlobatannins (Precipitate test)

Deposition of a red precipitate when 2 ml of extract was boiled with 1 ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

K. Test for Amino acids and Proteins (1% Ninhydrin Solution in Acetone).

2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

L. Test for Saponins (Foam test)

To 2 ml of extract was added 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

M. Test for Sterols (Liebermann-Burchard test)

1 ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red color.

N. Test for Tannins (Braymer's test)

2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish color solution.

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O. Test for Terpenoids (Salkowki's test)

1 ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

P. Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or coloration).

Table I: Physicochemical analysis of fruit of *Bombax ceiba*

S.No.	Parameters	Result (% w/w)
1	Total Ash	5.99
2	Acid insoluble ash	4.23
3	Water insoluble ash	2.22
4	Water soluble extractive value	13.16
5	Alcohol soluble extractive value	3.94
6	Loss on drying	9.57

Table II: Result of phytochemical evaluation of fruit of *Bombax ceiba.*

S.No	Phytochemicals in the plant extracts	Hexane	Methanol	Chloroform	Ethanol	Water
1	Alkaloids	+	+	+	+	+
2	Cardiac Glycosides	+	+	_	+	_
3	Carbohydrates	+	+	+	+	+
4	Flavonoids	+	+	_	+	_
5	Phenols	+	+	_	+	_
6	Phlobatannins	_	+	_	_	_
7	Proteins	+	+	_	+	+
8	Saponins	+	+	+	+	+
9	Sterols	_	+	_	_	_
10	Tannins	+	+	+	+	+
11	Terpenoids	+	+	_	+	_
12	Quinones	+	+	_	+	+

All the extracts of the fruit extract of Bombax were further analyzed for total Phenolic content, total Flavonoid content and total Antioxidative potential.

Q. Total Phenolic Content

Total phenolic content was measured according to Folin ciocalteu method [8]. A reaction mixture of 10 ml was made which comprised of 100µg/ml plant extract, 5ml Folin ciocalteu reagent, and 4ml 7% Na2CO3 which was mixed, and then incubated at 400 C in water bath for 30 minutes. After that, OD was taken at 760nm. Gallic acid of different concentration was taken as standard and the phenolic content was expressed as Gallic acid equivalents present per milligram of the dried plant extract sample.

R. Total Flavonoid Content

Aluminum chloride method was used for the determination of flavonoid content [9]. In 1 mg plant extract (0.5ml), 1.5 ml methanol was added. Then, 0.1 ml of the aluminum chloride (10%), 0.1 ml of the 1M potassium acetate, and 2.8ml the distilled water were added in the mentioned order and left at room temperature for 30 min. The absorbance of the mixture was taken at 415nm after incubation. Quercetin was used as standard, and the total flavonoid content of 1mg plant extract was determined and expressed in terms of quercetin equivalent.

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S. Determination of Total Antioxidant Activity

The total antioxidant activity of the extracts of *Bombax* was evaluated by using the phosphor-molybdenum method according to the procedure of Prieto *et al.*[10]. To 3ml of plant extract, 3ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The OD was measured at 695nm after incubation of the mixture at 900 C for 90 minutes. The antioxidant activity was expressed as the equivalents of ascorbic acid for 1milligram of the plant extract.

As shown in table 2 ,Methanolic extract showed maximum amount of phytochemicals , also there has been found the availability of flavonoids majorly ,therefore methanolic extract of the fruit was further tested for flavonoid contents using Thin Layer Chromatography (TLC) and different flavonoids were obtained depending on different RF value.

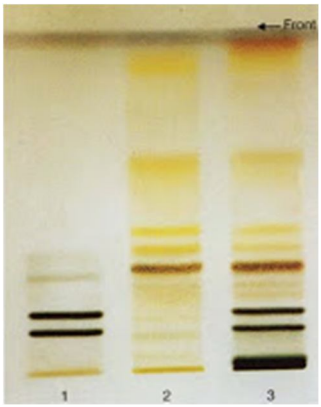

Table: III Colors can be observed at 365 nm UV light as follows:

S.No.	Compound	Color
1	Quercetin, myricetin and 3 & 7-O-glycosides	orange-yellow
2	kaempferol, isorhamnetin, and 3 & 7-O-glycosides	yellow-green
3	Luteolin and 7-O-glycosides	orange
4	Apigenin and 7-O-glycosides	yellow-green

Table IV : RF values of different flavonoids

S.No.	Flavonoids	RF Values	Color under UV 365 nm
1	Rutin	0.44	Orange
2	Hyperoside	0.65	Orange
3	Orientin	0.70	Yellow
4	Iso quercetin	0.72	Orange

III. RESULT AND DISCUSSION

Results for quantitative determination of proximate analysis and qualitative screening of phytochemicals in fruit of *B. ceiba* are presented in Table 1 & 2. Total ten phytochemicals were screened in which eight were found present in different solvent extracts. They are flavonoids, phenols, carbohydrates, saponins, tannins, alkaloids, proteins and terpenoids. Remarkably, carbohydrate flavonoids, phenols, saponins, tannin, quinones, alkaloids and terpenoids were present in the fruit of these plants. This suggests that the fruits have extensive potentials of phytochemicals.

Physiochemical parameters of the fruit of *Bombax ceiba* Linn.are tabulated in Table 1.

Different extracts of the powdered fruit were prepared for the study of extractive values. Percentage of extractive values was calculated with reference to the air dried drug. The results are shown in Table 1.Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant material can be easily deteriorated due to fungus. The loss on drying at 105°C in fruit was found to be 15.4 %. Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value content was 7.25 %. The negligible amount of acid insoluble siliceous matter present in the plant was 6.25 %. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids.

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In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, phenols shows different types of results in different solvents. From the fruit, water extract showed the presence of carbohydrate, alkaloids, saponins and tannins. However, 70% ethanol and acetone had the presence cardiac glycosides, carbohydrates, flavonoids, phenols, saponins, proteins, alkaloids and terpenoids. The methanol extract had the presence of cardiac glycosides, carbohydrate, alkaloids, flavonoids, phenol, tannins, saponins and terpenoids.[11].

The presence of phytochemicals in the extract of *P. niruri* was screened qualitatively. The result showed the presence of phenols, flavonoids, alkaloids, terpenoid and saponins. (Table 2). Phenols and flavonoids have significant antioxidant properties. Phenols are also associated with the ability to inhibit the growth of bacteria. Furthermore, these compounds have shown anti-inflammatory, anticancer and antidiabetic activity. The presence of these compounds formed the basis of further evaluation of the antioxidant and antimicrobial properties of the *Bombax ceiba* extract.

As, phenols and flavonoids are the secodary metabolites, and have antimicrobial and antioxidant activity, their presence was quantitatively determined. The extract of *semal* showed the presence of phenols and flavonoids both qualitatively and quantitatively. The analysis of the total phenol content showed the presence of total phenol content equivalent to 28.05 µg of gallic acid in 1mg of the plant extract. Flavonoids which are responsible for pigmentation in plants was also evaluated quantitatively. By the standard graph formed using quercetin, the flavonoid content in 1 mg of the plant extract was found to be equivalent to 61.41 µg of quercetin. The medicinal value of plants means definite physiological action on the human body due to presence of chemical substances. Different phytochemicals have been found to possess a wide range of activities, which might help in protection against diseases.

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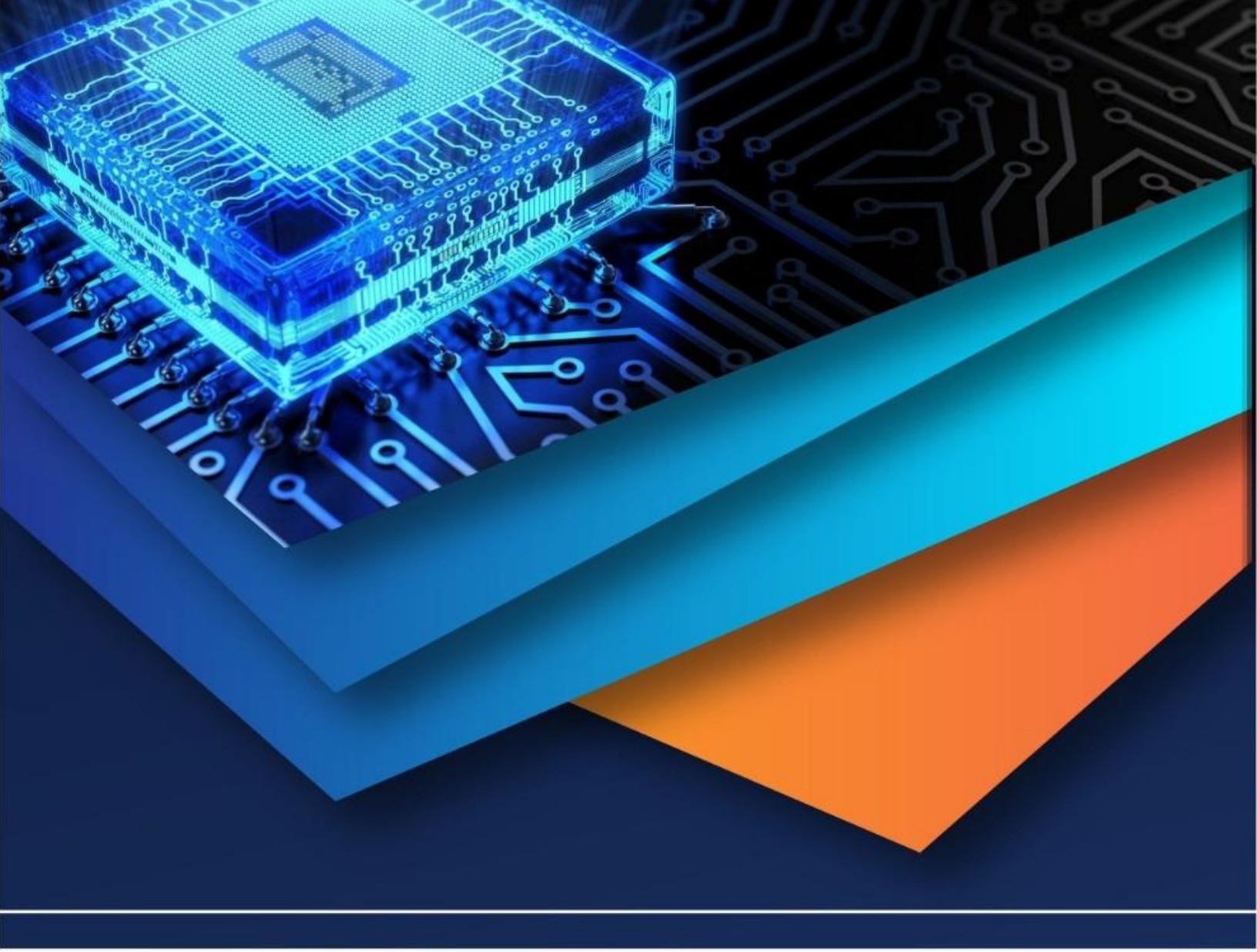

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