Phytochemical Screening of *Bombaxceiba* Flowers

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Abstract- Traditional knowledge of medicinal plants is showing important and significant values to society. *Bombaxceiba* is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. The present study includes the detailed exploration of phytochemical properties of various extracts of *Bombaxceiba* in an attempt to provide a direction for further research. Flowers of *Bombaxceiba* were air-dried and its coarsely powdered samples were subjected to Soxhlet extraction using diverse solvents (petroleum ether, chloroform , ethanol, ethyl acetate and distilled water)). Freshly prepared extracts were exposed to standard phytochemical analysis for qualitative estimation of phytoconsituents. Phytochemical analysis revealed the presence of several phytochemicals viz., alkaloids, flavonoids, steroids, phenol, tannins, steroid, terpenoids and glycocides. The methanolic extract displayed the presence of highest phytochemical compounds. It may be due to the higher solubility of active components in this solvent as compared to other solvents. The studies justify the use of *Bombaxceiba* in traditional medicines. The investigation further propose that the metabolites present in leaf tissue of *Bombaxceiba* can be potential source of novel natural antibacterial and antioxidant agents which may be of prospective application in food industry as an antioxidant.

Keywords: Phytochemical, *Bombaxceiba*, Antioxidant

Introduction

Since the ancient times, nature has been a huge source of medicinal plants. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine [1,2]. People are now showing interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs which have adverse side effects. Medicinal plants are mostly locally available, relatively cheaper and there is every virtue in exploiting such local and traditional remedies when they have been tested and proven to be non-toxic, safe, inexpensive and culturally acceptable to the community [3]. The medicinal value of plants is mainly due to the presence of some chemical substances known as phytochemicals. These are basically plant metabolites synthesized in all part of plant body by itself and have some definite physiological action on animals [4,5].

Bombaxceiba, commonly known as the Silk cotton tree belongs to the genus Bombax and family Malvaceae it is an important medicinal plant of tropical and sub-tropical India[6,7]. This tree is also found widely in tropical Asia, Africa and Australia[8]. The different parts of this plant have been used in the traditional system of medicines since ancient times[9]. The tree is famous for it's large, showy, six-inch flowers with thick, waxy, red petals that densely clothe leafless branch tips in late winter and early spring[10]. Many parts of the plant (root, stem bark, gum, leaf, flower, fruit, seed and heartwood) are mainly used by various tribal communities and forest dwellers for the treatment of wide variety of ailments[11]. Various activities have been reported in almost all parts of *Bombaxceiba*, some of these are hypertensive, antioxidant, hypoglycemic, antipyretic and hepatoprotective. The plant is used in traditional system of medicine as diuretic, dysenteric, emetic and curing diarrhoea, wounds, acne, skin blemish

and pigmentation, cold and cough [12].
The aim of this work was to determine the specifically phytochemical constituents of the floral extract of *Bombaxceiba*.

Material and Methods

The present study was carried at Post Graduate laboratory of department of Biochemistry and Biotechnology at Sardar Bhagwan Singh University, Balawala, Dehradun, India.

Sample collection and authentication

B. ceiba flowers were collected from campus of Sardar Bhagwan Singh University, Balawala, Dehradun in the month of January 2021 and authenticated from Botanical survey of India (BSI) Dehradun, (Uttarakhand), India.

Prepation of Plant extract

The plant material after collection was washed with distilled water to remove all fibrous and soil debris and then sun dried for 15 days. Dried sample was crushed into powder by electric blender (electric grinder).The fine powder (200gm sample extracted with 800ml of each solvent) was then subjected to Soxhletation by using different solvents in increasing order of polarity (Petroleum ether < Chloroform <Ethanol< Ethyl acetate< Distilled water). Different solvents were used for dissolving different components present in the plant material. The extract was then dried to remove almost all the moisture and solvents and thus the final product was kept in air tight containers and stored at 4°C in the refrigerator for further study.

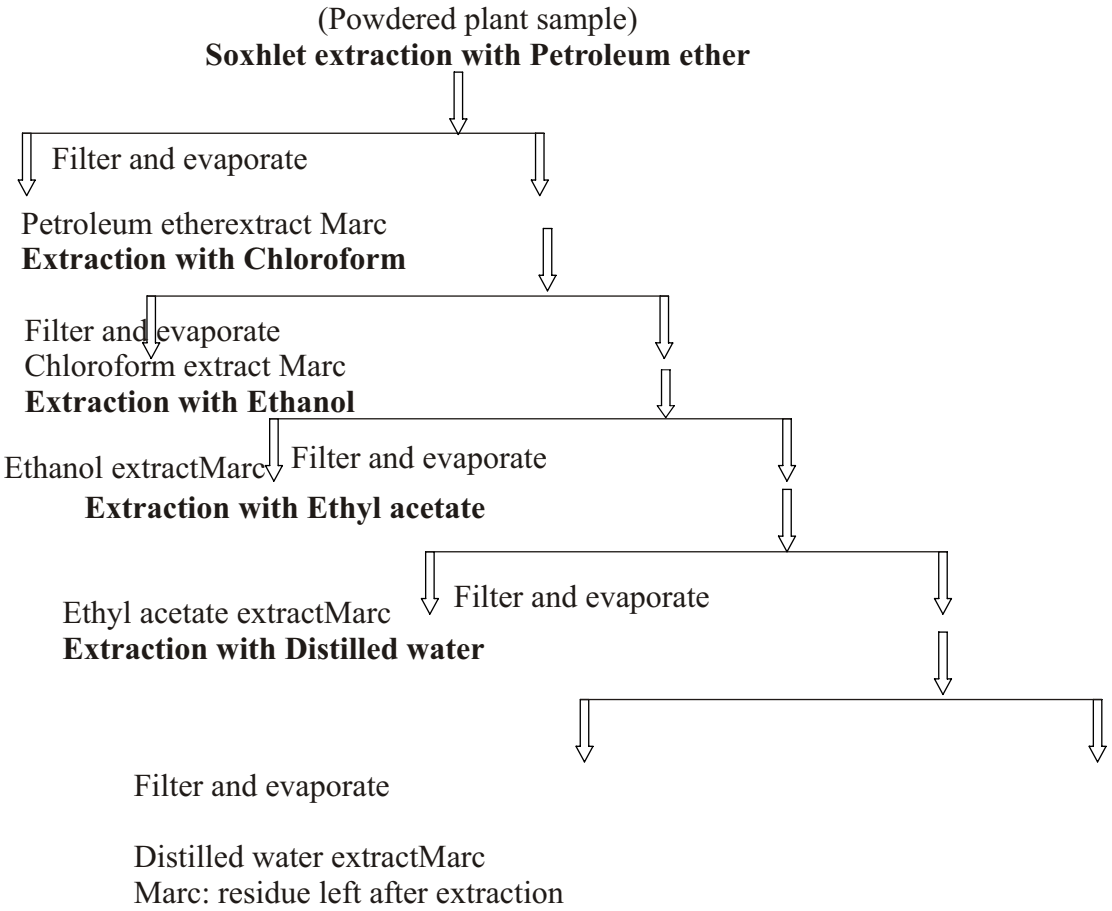

Figure -1 Scheme of *extraction*

Preliminary phytochemical investigation

All the extracts(Petroleum ether, chloroform extact, ethanolic extract, ethyl acetate extract and distilled water extract) obtained were subjected to preliminary phytochemical screening for the detection of various phytochemicals such as alkaloids, flavonoids, carbohydrate, steroids, phenols, tannins, saponins, terpenoid, glycoside, protein and amino acid using following standard methods[13-17].

Detection of Alkaloids -Small portion of the solvent free extract was stirred with a few drops of dil. HCL and filtered. The filtrate was then tested for following colour tests to detect the presence of alkaloid.

a. Mayer's reagent - Test solution with Mayer's reagent (1.36g of mercuric chloride in 60ml distilled water + 5.0g of potassium iodide in 20ml distilled water + 20ml of distilled water) gave cream ppt.

b. Hager's reagent - Test solution with Hager's reagent (saturated aq.solution of picric acid i.e. 1.0% w/w solution of picric acid in hot water) gave yellow ppt.

c. Wagner's reagent - Test solution with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 5ml of water and 100ml distilled water) gave reddish brown ppt.

Detection of Flavonoids

Alkaline reagent test: - Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomecolourless on the addition of dilute acid, indicated the presence of flavonoids.

Test for Carbohydrates

a. Molish's test - To the 2-3 ml of extract, few drops of 95% alpha-napthol solution in alcohol were added. After shaking, conc. H_2SO_4 was added from the sides of the test tube. Appearance of violet ring at the junction of two layers indicated the positive test for reducing sugar.

b. Fehling's test- To1 ml Fehling A and 1 ml Fehling B reagent 1 ml of extract was added and boiled for about 10min. Formation of brick red color precipitate indicated the presence of carbohydrate [18].

c. Benedict's solution test - Equal volume of Benedict's reagent and extract were mixed in test tube. Heated in boiling water bath for 5 min. Appearance of red coloured solution indicates the positive test for reducing sugar.

Test for steroids

a. Liebermann-Burchard Reaction - Mixed 2ml of extract with chloroform. Added 1-2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the sides of test tube. Development of green colour reveals the positive test for steroid moiety.

b. Salkowiski reaction - 2ml of crude extract was dissolved in 2ml of chloroform to this added 2ml of con. H_2SO_4 sidewise, red color ring was produced [19].

Test for phenolic components and tannins

Small quality of test solution dissolved in water and subjected for following test to detect the presence of phenolic compounds and tannins.

a. Dil. $FeCl_3$ solution (5%) test - Test solution with few drops of ferric chloride solution showed intense green color [20-23].

b. Vanillin HCl acid test solution - Test solution with vanillin reagent (1gm vanillin in 10 ml concentrated HCl) gave red color.

Test for saponins- Froth test: 2 ml of crude extract was mixed with 2ml of distilled water in a test tube, the solution was warmed and shaken vigorously; formation of stable foam indicated the presence of saponin.

Test for protein and amino acids

a. Ninhydrin solution test - Heated 3ml of extract and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 min. The development of violet or purple colour showedthe presence of amino acids[24].

b. Biuret test: - To 3ml of aqueous extract added 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour is formed, if proteins are present.

Detection of Glycosides

Extracts will be hydrolyzed with dilute HCl and then filtered. The filtrate obtained will be subjected to the following tests for glycosides.

a. Modified Borntrager's test- Extracts will be treated with 5 % Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was then 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).

b. Legal test- Extract as treated with sodium nitroprusside in Pyridine and NaOH. Formation of Pink to blood Red colour indicates the presence of glycosides (Cardiac glycosides).

Results and Discussion

Phytochemical Screening

Phytochemical screening of the sequential extract of *Bombaxceiba* revealed the presence of various bioactive components. The test for alkaloid has given positive result whereas saponin and protein test showed negative result for all the four extracts taken under study. Glycosides were present only in ethanol and ethyl acetate extract. Similarly phenolic compounds and tannins were present found in all the extracts except petroleum ether.

The result of phytochemical test is presented in Table -1.

Table- 1 Shows qualitative phytochemical analysis of extracts of *Bombaxceiba*

Phytochemical Test	Petroleum ether Extract	Chloroform Extract	Ethanol Extract	Ethyl acetate Extract	Distilled Water Extract
Test for flavonoids					
Alkaline reagent test	+	+	-	+	-
Test for Alkaloids					
Mayer's test	+	+	-	+	-
Hager's test	-	+	+	-	-
Wager's test	+	+	-	-	+
Test for carbohydrates					
Fehling test	+	-	-	+	+
Molish test	-	+	-	+	
Benedict's test	-	+	-	+	+
Test for phenolic compounds and tannins					
Dil. $Fecl_3$-test	-	+	+	+	+
Vanillin - HCl Test	_	+	+	+	+
Test for steroids					
Salkowiski test	+	+	+	-	-
Test for saponins					
Froth Test	-	-	-	-	-
Test for proteins					
Ninhydrin	-	-	-	-	-
Biuret test	-	-	-	-	-
Test for Terpenoids					
Salkowski test	+	+	+	-	-
Test for Glycosides					
Keller-Killiani test	-	-	+	+	-

(-) A sign indicates absence of constituent in the respective screening test; (+) sign indicates the presence of a constituent in the respective screening test.

These results are in confirmation with earlier studies done for this plant [25,26]. Flavonoids have extensive biological properties that promote human health and help in reduction of risk of diseases due to their antioxidant, anticancer, anti-inflammatory and anti-microbial properties[27]. Tannins are basically cytotoxic agents. They act as free radical scavengers thus can be useful in treatment of various degenerative diseases like cancer, atherosclerosis and aging process[28]. Alkaloids are being used in life saving drugs for some critical disorders like cancer, heart failure, blood pressure due to their wide range of pharmacological activities[29]. Saponins have been considered as bioactive antibacterial agent but also act as anti-tumour agents by inducing apoptosis [30].

Preliminary screening of phytochemicals is a valuable step in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. From the above results, it can be noted that successful extraction of biologically active compounds from plant are largely dependent on the type of solvent used during extraction. In this study, different solvents were used. This study, therefore, validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract.[31].

Conclusion

Herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and mainstream system. India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant based formulations, used in folk medicine and known to rural communities in India. Medicinal plants play a central role not only as traditional medicines, but also as trade commodities.

In the present work phytochemical and antimicrobial investigation of *Bombaxceiba* was performed. Successive solvent extraction was done using soxhlet. Preliminary phytochemical screening of *Bombaxceiba* gave valuable information about the different phytoconstituents present in the plant. It showed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins and amino acids. This will helps the future investigators in regard to the selection of the particular extract for further investigation of isolating the active principle and also will give idea about different phytochemicals, which have been found to possess a wide range of activities in *Bombaxceiba* stem extracts. Further studies on purification qualification and antioxidants potential of the active compounds would be our priority in future studies. Both in vitro and studies are in vivo recommended for their therapeutic application in modern medicine.

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