Environment and Ecology 42 (2) : 405—412, April—June 2024
Article DOI: https://doi.org/10.60151/envec/BFRU1901
ISSN 0970-0420

Quantitative, Antibacterial and Antioxidant Activity Screening of Various Phytoconstituents in *Bombax ceiba* Floral Extracts

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Received 10 July 2023, Accepted 12 January 2024, Published on 5 April 2024

ABSTRACT

The present study aims at investigations of quantitative, antibacterial and antioxidant activity of *Bombax ceiba* floral extracts. Preliminary qualitative phytochemical analysis was performed by following standard protocols. Total phenol and flavonoid content were evaluated by colorimetric method, using Folin- Ciocalteau Reagent and Aluminium Chloride, respectively. Antibacterial activity was evaluated by disc- diffusion method against *Staphylococcus aureus* and *Aeromonas* spp. Antioxidant activity was evaluated by DPPH scavenging assay. All the extracts of *B. ceiba* displayed presence of majority of phytochemicals. TPC and TFC was maximum in methanol (182.14- 439.23µg of GAE/mg) and ethyl acetate (285.25- 820.24µg of QE/mg) extract respectively. Maximum antibacterial activity was displayed by chloroform and ethyl acetate extract against *S. aureus* and *Aeromonas* spp., respectively. Antioxidant activity was maximum in aqueous extract and minimum in petroleum ether extract. The results suggests that *B. ceiba* is a rich source of phytochemicals. It has significant amount of phenols and flavonoids. It exhibits good antibacterial and antioxidant potential. Further studies can be carried out to find the components responsible for its various pharmacological activities. The extract of such beneficial plant can be a good source for the development of new drugs.

Keywords Antibacterial, Antioxidant, Phytochemicals, DPPH scavenging assay.

INTRODUCTION

India, with an abundance of medicinal plants, holds a strong knowledge of herbal system where Ayurveda is the oldest medicinal system (National Academy of Sciences. Biodiversity. Washington, DC 1998). Medicinal plants play an important role in pharmaceutical industries for their roles in disease prevention and treatment (Yu *et al.*2021). These medicinal properties in plants are attributed to phytochemicals that are basically plant secondary metabolites and have some definite physiological action on other living organisms (Giri *et al.* 2014, Tasneef *et al.*2013) They are present in abundant amount in medicinal plants and act as substrates for biochemical reactions, co-

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factors or inhibitors of enzymatic reactions, ligands that agonize or antagonize cell surface or intracellular receptors, scavengers of reactive or toxic chemicals, or compounds that enhance the absorption of essential nutrients. These compounds are claimed to be effective against cancers, coronary heart disease, diabetes, high blood pressure, microbial, viral, and parasitic infections, psychotic diseases, spasmodic conditions, ulcers (Dillard and German 2000). Based on chemical structures and characteristics, phytochemicals are categorized into six types that include lipids, phenolic, carbohydrates, terpenoids, alkaloids and other nitrogen-containing compounds (Vega and Oomah 2019).

Bombax ceiba is one such useful medicinal plant that belong to the family of Malvaceae and is widely distributed in tropical and subtropical India, Sri Lanka, Pakistan, Australia, Malaysia. It is commonly known as semul, cotton tree, red silk cotton tree and kapok (Raut *et al*. 2017). *B. ceiba* is a lofty, deciduous tree up to 40 m tall and 6m or more in girth with horizontally spreading branches and young stems covered with hard prickles. The tree is a strong light-demander, fast-growing and grows best on deep sandy loams or other well- drained soils, particularly in valleys (Parrotta 2001). The fruits are brown capsule-like up to 15 mm long, filled with numerous black ovoid- shaped seeds with dense silky hair. The tree is famous for its large, six- inch flowers with thick, waxy, crimson red to orange petals traced during late winter and early spring (Rameshwar *et al.* 2014). It has numerous traditional uses and its medicinal benefits have been reported in the Indian traditional systems of medicine (Donald *et al.* 2012). The extracts of *B. ceiba* species play an indispensable role in pharmacological properties including antibacterial, antioxidant, antidiabetic, hepatoprotective, antimicrobial, antianxiety, antipyretic (Ravi *et al.* 2010, Rehman *et al*. 2017, Mir *et al.* 2017, Wanjari *et al*. 2016, Shah *et al.* 2018). The tender bark is used as famine food, demulcent, emetic and tonic, and its aqueous extract mixed with curd is useful to check blood dysentery (Alsayari *et al*. 2018). The roots of very young tree possess aphrodisiac and astringent properties. The gum is also used as an astringent and in treating diarrhea and dysentery. The young fruits are dried and used as a demulcent and astringent in southern parts of India (Karole *et al*. 2018).

The tender twig is used as a toothbrush to cure mumps in some parts of India (Raw herbs of *Bombax ceiba.* http://www.la-medicca.com/rawherbsbombaxceiba.html). It is potentially effective against cold and cough, wounds, acnes, skin blemishes and pigmentation. This plant also exhibits diuretic and emetic properties (Chaudhary and Khadabadi 2012). Various tribal communities and forest dwellers rely on its roots, stem bark, leaves, flowers, fruits, seeds, gum. for the treatment of immense range of ailments (Verma *et al.* 2014, Verma *et al.* 2011).

Phenolic compounds contain one or more aromatic rings with single or multiple hydroxyl groups and can be classified in three main categories- Simple phenols, Polyphenols and miscellaneous. These compounds can be found in a free state, conjugated with sugars or esters, or polymerized. There is a growing interest in the quantification of phenol content in medicinal plants due to their effectiveness against a bunch of chronic illnesses (Mujica *et al.* 2009).

The generation of ROS in the body leads to physiological disorders such as glycated protein oxidation in diabetes mellitus, red blood cell hemolysis in glucose-6-phosphate dehydrogenase deficiency. Many plants like *B. ceiba* contain substantial amounts of antioxidants like vitamin C and E, carotenoids, flavonoids, tannins that can be used to scavenge the excess free radicals from human body (Gandhare *et al.* 2010).

Taxonomic position of *Bombax ceiba*

Kingdom- Plantae
Subkingdom- Tracheobionta
Super division- Spermatophyta
Division- Magnoliophyta
Class- Magnoliopsida
Subclass- Dilleniidae
Order- Malvales
Family- Malvaceae
Genus- *Bombax*
Species- *ceiba* L.

Common name- English: - Red silk cotton tree,

kapok, silk cotton tree.

MATERIALS AND METHODS

Sample collection and authentication

B. ceiba flowers were collected from the campus of Sardar Bhagwan Singh University, Balawala, Dehradun in the month of February, 2022 and subjected to authentication from Botanical Survey of India (BSI) Dehradun, (Uttarakhand) with Accession No. 1147, dated 29 July, 2022 Soxhlet Extraction.

The plant material (200 g) was washed with distilled water to remove soil debris and then shade dried for 15-20 days. The dried sample was then crushed into fine powder by electric blender, that along with 800 ml of each solvent, was then subjected to Soxhlation using different solvents in increasing order of polarity (petroleum ether< chloroform< ethyl acetate< methanol< water) (Donipati *et al.* 2014). The final products thus obtained was kept in an air- tight container and stored at 4°C in the refrigerator for further studies.

Qualitative phytochemical analysis

All the extracts were subjected to preliminary phytochemical screening, using standard methods for the detection of various phytochemicals. Different extracts of *B. ceiba* were subjected to phytochemical screening for the following phytochemicals- alkaloids, flavonoids, carbohydrates, steroids, phenols, tannins, saponins, terpenoids, glycosides, proteins, and amino acids (Sukumaran *et al.* 2011, Kokate 1994).

Estimation of total phenol content (TPC)

Different concentrations of methanol and aqueous extract were separately mixed with 1ml of Folin- Ciocalteau phenol reagent and 0.8 ml of aqueous 20% Na_2CO_3 solution and allowed to stand for 15 minutes. Then, absorbance at 765 nm was measured using UV-visible spectrophotometer. The sample concentration was calculated with the help of standard plot of Gallic acid (25-250 µg/ml) and TPC was expressed in terms of µg of the Gallic acid equivalent (GAE) per mg of the dry mass (Fig.1) (Sharma and Kalauni 2017).

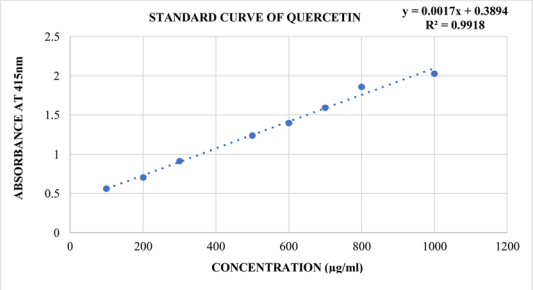

Fig 1. Standard curve of quercetin for estimation of total flavonoid content.

Estimation of total flavonoid content (TFC)

Different concentrations of ethyl acetate and methanol extract were separately mixed with 0.75 ml of methanol, 0.05ml of 10% $AlCl_3$, and 0.05 ml of 1M potassium acetate and 1.4 ml of distilled water and allowed to stand for 30 minutes. The absorbance at 415nm was measured using UV- visible spectrophotometer and the sample concentration was calculated using standard plot of quercetin (10-100 µg/ml). The TFC was expressed in µg of quercetin equivalent (QE) per mg of the dry mass (Sharma and Kalauni 2017).

Evaluation of antibacterial activity

In- vitro antibacterial activity was determined by disc diffusion method against a Gram- positive bacterium, *Staphylococcus aureus* and a Gram- negative bacterium, *Aeromonas* spp. They were obtained from the Microbiology Department of Sardar Bhagwan Singh University, Balawala. The bacterial strains were identified by staining and morphological characteristics. The nutrient agar medium was prepared, followed by autoclaving at 121°C for 15 minutes and then allowed to solidify on Petri plates. Meanwhile, each plant extract was separately dissolved in Dimethyl Sulfoxide (DMSO) to obtain different concentrations i.e., 100µl/ml, 200µl/ml, 300µl/ml, and 500µl/ml. The solidified plates were inoculated with 100 µl of suspension culture of both the bacterial strains. Later, the paper discs saturated with each extract of different concentrations, were placed on respective section of each plate. The plates were incubated in

upright position at 37°C for 24 hrs. Any zone of inhibition around the well indicated the presence of antibacterial activity.

Evaluation of antioxidant activity

Antioxidant activity was evaluated by DPPH scavenging assay 2,2-diphenylpicrylhydrazyl (DPPH) solution was prepared by mixing 15mg of DPPH in 10 ml of methanol. 75µl of this solution was diluted with methanol to obtain a final volume of 3ml. The absorbance was taken immediately at 517nm for control reading. Later, 75µl of DPPH solution was added to the tubes containing different concentrations of each sample i.e., 100µl, 300µl, 500µl, and 750µl. The mixture was then diluted to 3ml with methanol. Decrease in absorbance was then measured at 517nm. Ascorbic acid was used as a reference. The results were expressed as a percentage inhibition of DPPH calculated from the following formula-

% Radical inhibition= ([Ao- A1]/ Ao) × 100 Where,

Ao= Absorbance of blank

A1= Absorbance of sample

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Various phytochemicals such as alkaloids, phenols, tannins, carbohydrates, proteins, flavonoids, saponins were found in floral extracts of *B. ceiba*. The ethyl acetate extract displayed positive results for most of the phytochemicals whereas aqueous extract displayed positive results for few phytochemicals (Table 1). The results are in agreement with the findings of Hait and Goswami (2017), that reported the presence of majority of phytochemicals in different extracts of the plant (Verma *et al.* 2014).

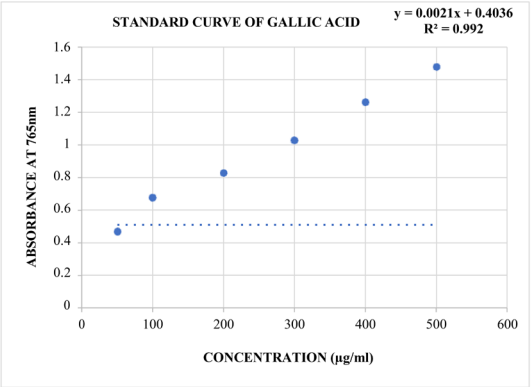

Fig. 2. Standard curve of gallic acid for estimation of total phenol content.

Table 1. Results of qualitative phytochemical analysis of different extracts of *B. ceiba* flowers.
A minus (-) sign indicates a negative result for the test while a plus (+) sign indicates a positive result.

Test performed	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Mayer's test	+	+	+	-	+
Hager's test	-	+	+	-	-
Wagner's test	+	+	+	-	+
Alkaline reagent test	-	+	+	+	-
Ammonia test	-	-	+	+	-
Ferric chloride test	-	-	-	+	+
Molisch's test	+	+	+	+	+
Fehling's test	+	-	+	+	+
Benedict's test	+	+	+	-	+
Salkowski test	+	+	+	-	-
Vanillin-HCl test	-	-	-	+	+
Foam height test	+	+	+	+	-
Bontrager's test	-	+	+	-	-
Legal test	-	-	+	+	-
Millon's test	-	+	-	+	-
Biuret's test	-	-	+	+	+
Ninhyrdin test	-	-	-	+	+

Estimation of TPC

Based on Fig. 2, phenol content of *B. ceiba* floral ex-

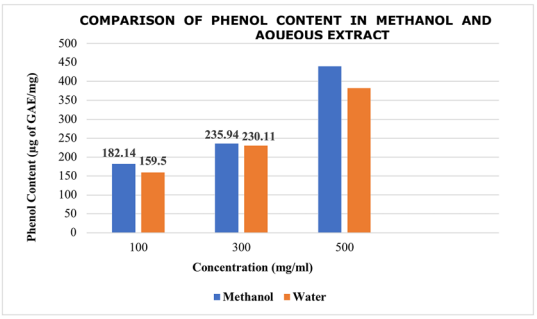

Fig. 3. Comparison of TPC in methanol and aqueous extract of *B. ceiba* flowers.

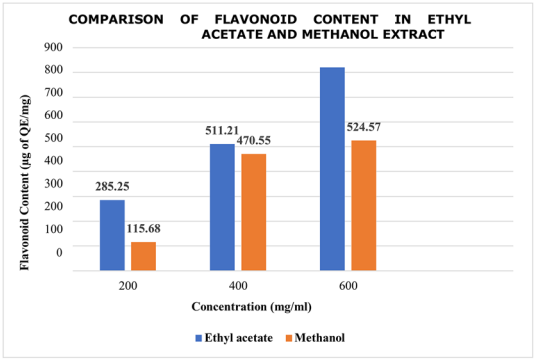

Fig. 4. Comparison of TFC in ethyl acetate and methanol extract of *B. ceiba* flowers.

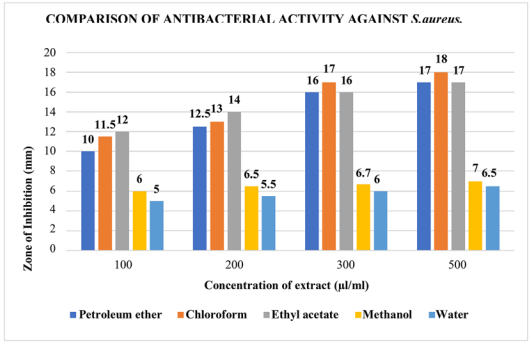

Fig. 5. Comparison of antibacterial activity of different extracts of *B. ceiba* against *S. aureus.*

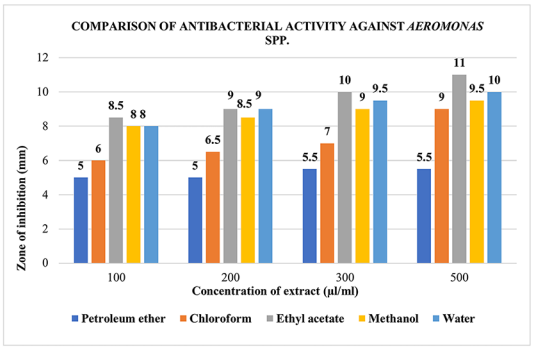

Fig. 6. Comparison of antibacterial activity against *Aeromonas* Spp.

tracts was calculated in methanol and water extract of different concentrations (100 mg/ml, 300 mg/ml, and 500 mg/ml). The phenol content of both the extracts is depicted in Table 2. The results suggested that the methanol extract has more phenol content than aqueous extract (Fig. 3). In comparison to the findings of Singh *et al.* (2016), phenol content in the present study was reported much greater, ranging from 182.14 to 439.23µg of GAE/mg in methanolic extract and from 159.50 to 382.0523µg of GAE/mg in aqueous extract. It suggests that phenol content is in high amount in the present sample (Verma *et al.* 2011).

Table 2. Total phenol content of methanol and aqueous extract of *B. ceiba* flowers.

Concentration of extract (mg/ml)	Phenol content of extracts (µg of GAE/mg)	
	Methanol	Aqueous
100	182.14	159.5
300	235.94	230.11
500	439.23	382.05

Estimation of TFC

Based on Fig. 4, flavonoid content of *B. ceiba* floral extracts was calculated in ethyl acetate and methanol extract of different concentrations (200 mg/ml, 400

Table 3. Total flavonoid content of ethyl acetate and methanol extract of *B. ceiba* flowers.

Concentration of extract (mg/ml)	Flavonoid content of extracts (µg of QE/mg)	
	Ethyl acetate	Methanol
200	285.25	115.68
400	511.21	470.55
600	820.24	524.57

Table 4. Antibacterial activity of different extracts of *B. ceiba* against *S. aureus* and *Aeromonas* spp.

	Zone of inhibition (mm)							
	Staphylococcus aureus				*Aeromonas* spp.			
Concentration of extracts (µl)	100	200	300	500	100	200	300	500
Petroleum ether	10	12.5	16	17	5	5	5.5	5.5
Chloroform	11.5	13	17	18	6	6.5	7	9
Ethyl acetate	12	14	16	17	8.5	9	10	11
Methanol	6	6.5	6.7	7	8	8.5	9	9.5
Aqueous	5	5.5	6	6.5	8	9	9.5	10

mg/ml, and 600 mg/ml). The flavonoid content of both the extracts is depicted in Table 3. The results suggested that the ethyl acetate extract has more flavonoid content than methanol extract. Singh *et al.* (2016) reported a fair amount of flavonoid content in their findings ranging from 3.17 to 102.2µg of QE/mg. Meanwhile, present study reported a significant amount of flavonoid in the both the sample, ranging from 285.25 to 820µg of QE/mg in ethyl acetate extract and from 115.68 to 524.57µg of QE/mg in methanolic extract (Verma *et al.* 2011).

Evaluation of antibacterial activity

Different concentration (100µl/ml, 200µl/ml, 300µl/ml, and 500µl/ml) of each extracts displayed zone of inhibition (ZOI) of different sizes. Maximum antibacterial activity was displayed by chloroform extract, followed by ethyl acetate extract against *S. aureus,* whereas minimum activity was exhibited by

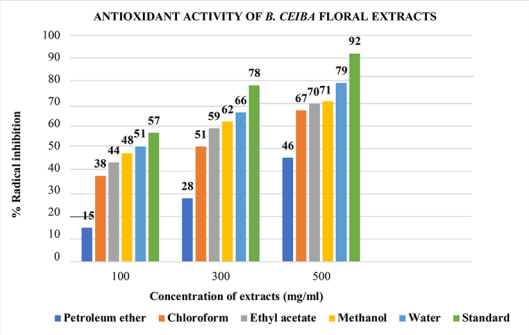

Fig. 7. Comparison of antioxidant activity of different extracts of B. ceiba flowers.

Table 5. Antioxidant activity of *B. ceiba* floral extracts in terms % radical inhibition.

	% Radical Inhibition					
Concentration of extract (mg/ml)	Standard	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
100	57	15	38	44	48	51
300	78	28	51	59	62	66
500	92	46	67	70	71	79

chloroform extract against *Aeromonas* spp. Maximum activity against *Aeromonas* spp. was displayed by ethyl acetate extract followed by aqueous extract (Figs. 5 - 6). Digge *et al.* (2015) reported significant antibacterial activity in aqueous extract of *B. ceiba*. Where ZOI ranged from 15 to 32 mm against *S. aureus*. Meanwhile, the present study reported maximum activity in chloroform extract, where ZOI ranged from 11.5 to 18mm against *S. aureus* (Table 4).

Evaluation of antioxidant activity

Table 5 revealed the antioxidant activity of different concentrations of each extract. Maximum activity was displayed aqueous extract, followed by ethyl acetate and methanol extract. Meanwhile, minimum activity was displayed by petroleum ether extract. Chloroform extract exhibited moderate scavenging activity. The percent radical inhibition was directly proportional to the concentration of extract (Fig.7). The results were in accordance with the reports of Chikatipalli (2021), who also reported significant antioxidant potential in *B. ceiba.*

CONCLUSION

Ayurveda is the most ancient yet living traditions that holds a strong knowledge of medicinal plants and their importance. The medicinal plants play vital role in disease prevention since they are rich source of phytochemicals.

The present work encompasses phytochemical

screening, estimation of TFC and TPC, and evaluation of antibacterial and antioxidant activity of *B. ceiba* flowers. The phytochemical screening revealed the presence of variety of phytochemicals. The phenol content was found in great amount in water extract, whereas the flavonoid content was maximum in ethyl acetate extract. *B. ceiba* exhibited good antibacterial and antioxidant potential. The antioxidant potential, in terms of DPPH scavenging potential, was found maximum in water extract, followed by methanol and ethyl acetate extract. Maximum antibacterial activity was reported in chloroform extract against *S. aureus* and in ethyl acetate extract against *Aeromonas* spp.

The phytochemical profiling and antioxidant activity provide a promising area of research in natural therapeutics and further studies can be carried out to find the components responsible for its various pharmacological activities. There is a need to find out novel agents with therapeutic properties to rule out the adverse side effects of conventional medicine system. Investigations of antibacterial activity is a powerful tool in the field of drug development. The extracts of such medicinal plants can be a good source for the development of new drugs. Subsequently, dose-limiting toxicity of drugs developed, can be studied.

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