Algerian Journal of Natural Products 7:1 **(2019)** 651- 656

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products

www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Phytochemical Screening, Antioxidant and Antibacterial Activities of *Bombax ceiba* Flower

Deepshikha Rathore[1], Richa Srivastava[1], Geetanjali[2], and Ram Singh[1*]

[1]Department of Applied Chemistry, Delhi Technological University, Delhi-110 042, India
[2]Department of Chemistry, Kirori Mal College, University of Delhi, Delhi - 110 007, India
ramsingh@dtu.ac.in

Received: 16/03/2019 /Accepted: 27/07/2019

Abstract: *Bombax ceiba* (Bombacaceae) is a well-known plant in the traditional systems of medicine. This plant species bears beautiful red coloured flowers from February to April. To utilize this flower as a potential source for value-added products, a qualitative phytochemical screening along with antioxidant and antimicrobial studies have been performed. The phytochemical screening revealed the presence of phenolics, tannins, amino acids and alkaloids in the methanolic extract of the flower. The antioxidant activity (AA) was evaluated using DPPH method. Some of the semi-purified extracts showed >90% AA. The MIC value for the extract was found to be between 3.125-12.500 µg/mL.
Keywords: *Bombax ceiba*, Flower, Phytochemical screening, Antioxidant activity, Antimicrobial activity

I. Introduction

Bombax ceiba belongs to the genus *Bombax* and family Bombacaceae which is an important medicinal plant of tropical and sub-tropical India.[1,2] This tree also found widely in tropical Asia, Africa and Australia.[3] The different parts of this plant have been used in the traditional system of medicines since ancient times.[4] The plant has also been evaluated for a number of pharmacological activities like HIV, diarrhoea, dysentery, urinary troubles, bladder disorders, gynaecological problems, heart diseases, and other medicinal properties.[2,5-9]

Bombax ceiba bears beautiful red coloured flowers from February to April. Most of these flowers go waste and hence a value addition is a requirement. The present work is a step towards value-addition to the *B. ceiba* flowers through their antioxidant and antimicrobial studies and a qualitative phytochemical screening.

II. Materials and Methods

II.1. *Plant material collection and sample preparation*

The flowers of *B. ceiba* were collected from the campus of Delhi Technological University (DTU), Bawana Road, Delhi – 110042, India from February to April. These were air dried in shade for 15 days at room temperature and powdered. This was extracted with methanol (1.5 kg in 3 L) in Soxhlet extractor using a round bottom flask (5 L) for 24 hours. The extract was filtered and the

solvent, methanol was removed under reduced pressure to get the crude extract (73 g) for further investigation as the original extract.

II.2. *Phytochemical screening*

The presence of a different group of phytocompounds was screened using the reported standard protocol.[10] The results are summarized in table 1.

II.3. *Antioxidant study*

The antioxidant study was performed for three different sets of extracts using reported DPPH (2,2 diphenyl-1-picryl hydrazyl) method.[11] Each extract from Set I-III (1 mL) was mixed with freshly prepared DPPH solution (3 mL) and allowed to react for 45 minutes at room temperature in dark. After that, the mixture was tested for DPPH radical scavenging activity on double beam UV-visible spectrophotometer at 517 nm. The solution of DPPH in ethanol (1.2 mg in 50 mL) was used as blank and studied at the same wavelength. The 0.6 mg of flower extract in 25 mL of ethanol was used as reference. As positive control gallic acid was used. The samples were run in triplicate and the mean value of three of them was recorded and results are given in table 2. Percentage of antioxidant activity was calculated using the formula:

$$AA(\%) = [(A_b - A_s) / A_b] \times 100$$

where AA = Antioxidant activity; A_b = Absorbance of blank; A_s = Absorbance of sample

Set I: The original methanolic flower extract (section 2.1) (100 mg) was mixed with 25 mL of different solvents (hexane, benzene, chloroform, ethyl acetate, acetone, methanol, and ethanol) and stirred at 25°C for 1½ h on water bath (50°C). The extract mixture was filtered and the filtrates were used for antioxidant studies.

Set II: The 5 mL of each solution from set I was subjected on water-bath to evaporate the solvent. To this extract, 1 mL of ethanol was added and shook to dissolve the same filtered and used for antioxidant.

Set III: The original methanolic flower extract (section 2.1) (100 mg) was mixed with 25 mL of hexane and stirred at 25°C for 1½ h on water bath (50°C). The solvent was filtered and the residue was again mixed with benzene (25 mL), stirred at 25°C for 1½ h on water bath (50°C). This process was repeated for chloroform, ethyl acetate, acetone, methanol, and ethanol respectively. The filtrates were used for antioxidant studies.

II.4. *Antimicrobial susceptibility screening*

The different groups of microorganisms such as *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative) as well as *Staphylococcus aureus* I, *Staphylococcus aureus* II and *Bacillus subtilis* (gram-positive) were used for antimicrobial activity. The antimicrobial screening was done in Laminar Hood with all the precautions including sterilization by autoclaving at a temperature of 121°C and a pressure of 15lbs./sq. inch for 20 minutes.

Mueller-Hinton Agar (9.00 g and 13.75 g) were dissolved in distilled water (250 mL) in Erlenmeyer flasks (500 mL). The pH of the medium was adjusted to 7.3. The flasks were plugged with non-absorbent cotton and sterilized in the autoclave at 15 psi for 20 minutes. The media (15 mL) was poured in Petri plates. The respective seed media were inoculated with cells from the surface growth of 1-day old slant of different bacteria with the help of a sterile inoculating loop wire aseptically. Then, the petri plates were placed in an incubator at 37°C for 24 h. The suspensions of the test organism were prepared by the following steps:

a) One loop full of culture was taken from the lawn culture and then smeared on the wall of test tube of normal saline (0.85% NaCl) and mixed it.
b) The opacity of the above solution was matched with the standard cell suspension (10^6 cell/mL). After that, the swab was taken and dipped into the test tube of normal saline and remove excess saline by pressing the swab on the wall of the test tube.

Zone of inhibition determination: The zone of inhibition determination was done by Kirby- Baur's method.[12,13] The stock solution was prepared in water/methanol and was serially doubly diluted (200 µg/mL: 100, 50 and 25 µg/mL). These solutions were poured over sterilized filter disks and disks were subsequently dried to remove excess solvent. Different bacterial strains (200 µL) selected along with culture broth were added on the plates and spread with the help of sterile spreader. The soaked filter paper disks were placed aseptically over the inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 h, in upright position. The zone of inhibition was measured using scale and results are reported in table 3.

Determination of MIC: The minimum inhibitory concentration (MIC) values were determined as per the standard protocol.[12,14] The stock solution of 200 µg/mL was prepared in water and was serially diluted as 100, 50, 25, 12.5, 6.25, 3.125 µg/mL. Different concentrations were prepared in sterile dry test tubes to determine MIC. The nutrient broth was prepared and 2.9 mL of it was taken in each test tube and were sterilized after plugging. After cooling, 0.1 mL of each dilution was added to the test tubes and the final volume was made up to 3.0 mL. To each test tube 0.1 mL of bacterial culture broth was added to bring the turbidity level to 0.5 McFarlands. The test tubes were shaken to mix the inoculums with the broth uniformly. The tubes were incubated at 37°C for 24 h and observed for any visible turbidity. The lowest concentration, at which no growth of microorganism observed, was termed as MIC and results were summarized in table 4.

III. Results and Discussion

III.1. *Phytochemical screening of B. ceiba flowers methanol extract*

Phytochemicals are bioactive chemicals of plants and regarded as secondary metabolites.[15-17] The screening for the presence of a phytochemical group helps in its further isolation, purification, and characterization. The phytochemical screening of the methanolic extract of *B. ceiba* flowers has been done according to standard literature procedure by using specific reagents.[10] The qualitative screening results showed the presence of alkaloid, phenolics, tannins, amino acids and proteins in the methanolic flower extract (Table 1). This result suggests that the flower of *B. ceiba* has the potential to be used in drugs and nutraceuticals. However, the tests for other phytochemical groups like carbohydrates, saponins, and sterols gave negative results (Table 1). The isolation of individual phytocompounds is in progress.

Table 1: *Phytochemical screening of methanol extract of B. ceiba flower*

S. No.	Test	Chemicals used	Observation	Inferences
1.	Molisch test	1-Naphthol in ethanol, H_2SO_4	No red to violet color ring appears at the junction	negative
2.	Ferric chloride test	Aqueous ferric chloride	Green color observed	positive
3.	Foam test	Water	No foam is observed	negative
4.	Libermann-Buchard test	$CHCl_3$, acetic anhydride, conc. H_2SO_4	No pink or red colour appeared	negative
5.	Braymer test	Alcoholic ferric chloride	Greenish colour appeared	positive
6.	Ninhydrin test	1% Ninhydrin in acetone	Purple color observed	positive
7.	Mayer's Test	Mayer's reagent	creamy ppt. observed	positive

III.2. *Antioxidant study*

The antioxidant activity (AA) of different solvent fractionated crude extracts of the flower obtained from the original methanol extracts was evaluated spectrophotometrically following the DPPH method (Figure 1, Table 2).[11,18,19]

The objective of the sample preparation (set I-III) was to identify the individual crude fraction possessing better AA. These factions are semi-purified extracts. The AA for original methanol extract was found to be 73% which is low in comparison to individualized factions. This may be due to the presence of both radical scavenging and promoter type of compounds. However, the AA increases in semi-purified fractions inferring the separation of phytocompounds towards better AA. The highest antioxidant activity (>90%) among the analyzed extracts were shown by polar solvent fractions like acetone, methanol, and ethanol for the set I, methanol and ethanol for set II and set III. They are found to be closure to the gallic acid. These fractions may be utilized in nutraceuticals and taken up for further investigations.

Table 2: *Antioxidant activity (AA%) of methanol extract of B. ceiba flower*

Exp	Hexane	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol	Ethanol	A_b
Set I	84.09	89.77	92.04	89.77	94.31	95.45	94.31	0.88
Set II	80.27	88.65	89.73	89.38	89.49	91.79	94.16	0.91
Set III	82.90	85.19	83.90	86.76	84.34	93.66	94.70	0.98

Gallic acid: 95.56; Range of UV-visible spectrophotometer: 400 to 650 nm

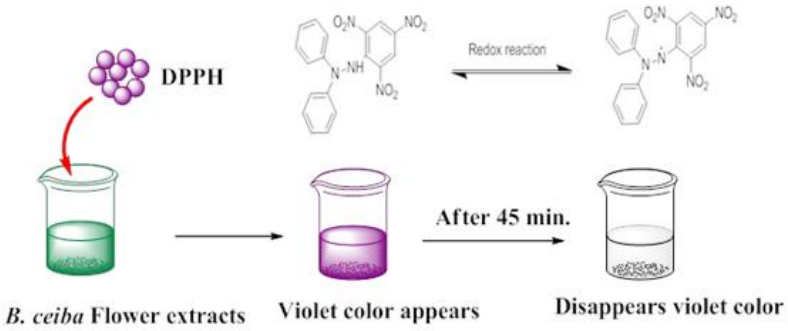

Figure 1*. Antioxidant evaluation*

III.3. *Antimicrobial susceptibility screening*

The antibacterial susceptibility test was done by determining the zone of inhibition by Kirby-Baur's method. The zone of inhibition was measured using scale (Table 3). The size of the zone of inhibition indicates the level of sensitivity. The methanol extract exhibited potent activity against both gram-negative and gram-positive bacteria. The results revealed significant antibacterial activity except for *Staphylococcus aureus II*. The activity against *S. aureus II* is inferior in comparison to the gram-negative bacteria. The possible reason for activity against gram-positive can be due to the fact that gram-positive bacteria are surrounded by a thick peptidoglycan layer or cell wall. It has little resistance to the diffusion of small molecules. The molecules reported here might diffuse easily through the loose outer wall of the gram-positive bacteria. Whereas, the pathogenic ability of gram-negative bacteria is usually associated with certain components of gram-negative cell walls, in particular, the lipopolysaccharide (LPS) layer. They have narrow porins or channels through which the newly synthesized semi-synthetic molecules might diffuse easily and can show antibacterial activity. Enhancement in activity of all the molecules can also be due to the higher cellular uptake and thus enhanced bioavailability of the drug molecules. The extract was further analyzed by the broth dilution assay to determine its MIC values as per standard protocol. The lowest concentration, at which no growth of microorganism observed, was termed as MIC. The results are presented in table 4. The extract was showing the antibacterial activity of MIC ranging between 3.125-12.500 µg/mL. The results suggest their application as antibacterial agents.

Table 3: *Antibacterial activity of methanolic extract of flower of B. ceiba*

Sample	Conc (μg/mL)	Zone of Inhibition (mm)					
		Gram-negative			Gram-positive		
		K. pneumoniae	*E. coli*	*P. aeruginosa*	*S. aureus* I	*S. aureus* II	*B. subtilis*
Flower extract	25	8	7	3	7	-	8
	50	11	8	5	10	-	10
	100	15	14	7	14	2	13
	200	20	16	8	16	5	18
Vanco-mycin	25	10	8	5	10	4	10
	50	12	11	8	13	5	13
	100	15	13	9	15	8	15
	200	24	22	15	22	10	22

Table 4: *The MIC determination of flower extract for different organisms*

Sample (μg/mL) → **Bacteria**↓	200	100	50	25	12.500	6.250	3.125
K. pneuminoae	X	X	X	X	X	X	√
E. coli	X	X	X	X	X	X	√
P. aeruginosa	X	X	X	X	√	√	√
S. aureus I	X	X	X	√	√	√	√
S. aureus II	X	X	X	X	√	√	√
B. subtilis	X	X	X	X	X	X	√

X: No growth; √: Growth

IV. Conclusion

The results suggest that the *Bombax ceiba* flowers are rich in alkaloid, phenolics, tannins, amino acids and proteins. It is a potential source of antibacterial, and antioxidant molecules. The flowers can be used as natural antioxidants, nutraceuticals, and preservatives in food and non-food systems and hence utilized in value addition to the products. However, further phytochemical studies are required to authenticate the presence of individual bioactive molecules.

V. Acknowledgement

The authors are thankful to DTU for instrumental facility. DR is thankful to DTU for financial assistant.

VI. References

[1] Vaidyaratnam, P.S. Indian Medicinal Plants - A compendium of 500 species, 4, Orient Longman publishing, Kottakkal, 1997, 391-395.

[2] Rathore, D.; Geetanjali; Singh, R. Phytochemistry and pharmacology of genus *Bombax. Nat. Prod. J.* (2019) DOI: 10.2174/2210315508666180831095836.

[3] Rani, S.; Rahman, K.; Sultana A. Ethnomedicinal and pharmacological activities of *Mochrus (Bombax ceiba* Linn.): An overview. *TANG* 6 (2016) e2:1-9.

[4] Jain, V.; Verma, S.K.; Katewa, S.S.; Anandjiwala, S.; Singh, B. Free radical scavenging property of *Bombax ceiba* Linn. Root. *Res. J. Med. Plant* 5 (2011) 462-470.

[5] Gandhare, B.; Soni, N.; Dhongade, H.J. *In vitro* antioxidant activity of *Bombax ceiba*. *Int. J. Biomed. Res.* 1 (2010) 31-36.

[6] Verma, V.; Jalalpure, S.S.; Sahu, A.; Bhardwaj, K.; Prakesh, Y. *Bombax ceiba* Linn: Pharmacognostical, phytochemistry, ethnobotany, and pharmacology studies. *Int. Pharma. Sci.* 1 (2011) 62-68.

[7] Anandarajagopal, K.; Anbu, J.S.J.; Promwichit, P. *Bombax ceiba* Linn. bark extracts show anti-microbial activity. *Int. J. Pharm. Res.* 3 (2011) 24-26.

[8] Jain, V.; Verma, S.K.; Katewa, S.S. Effect of *Bombax ceiba* root on some cardiovascular risk parameters in patients with ischemic heart disease. *Asian J. Biol. Sci.* 5 (2012) 351-357.

[9] Chaudhary, P.H.; Rai, P.D.; Deore, S.L.; Khadabadi, S.S.; Pharmacognostical and phytochemical studies on roots of *Bombax ceiba* Linn. *J. Pharm. Pharmacogn. Res.* 2 (2014) 172-182.
[10] Gokhale, S.B.; Kokate, C.K. *Practical Pharmacognosy*, Twelveth Ed., Nirali Prakashan, Pune, 2008.
[11] Wahab, S.; Hussain, A.; Farooqui, A.H.A.; Ahmad, Md. P.; Hussain, Md. S.; Rizvi, A.; Ahmad, Md. F.; Ansari, N.H. *In vivo* antioxidant and immunomodulatory activity of *Bombax ceiba* bark-focusing on its invigorating effects. *Am. J. Adv. Drug Delivery* 2 (2014) 1-13.
[12] Srivastava, R.; Bhargava, A.; Singh, R.K. Synthesis and antimicrobial activity of some novel nucleoside analogues of adenosine and 1,3-dideazaadenosine. *Bioorg. Med. Chem. Lett.* 17 (2007) 6239–6244.
[13] Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Path.* 45 (1966) 493–496.
[14] Brantner, A.; Grein, E. Antibacterial activity of some plant extracts used externally in traditional medicine. *J. Ethnopharcol*. 44 (1994) 35-40.
[15] Singh, R.; Geetanjali. *Asparagus racemosus*: a review on its phytochemical and therapeutic potential. *Nat. Prod. Res.* 30 (2016) 1896-1908.
[16] Fatima, A.; Singh, R. The chemistry and pharmacology of genistein. *Nat. Prod. J.* 6 (2016) 3-12.
[17] Singh, R.; Geetanjali. Phytochemical and pharmacological investigations of *Ricinus communis* Linn. *Alg. J. Nat. Prod.* 3 (2015) 120-129.
[18] Vieira, T.O.; Said, A.; Aboutabl, E.; Azzam, M.; Creczynski, P.; Tania B. Antioxidant activity of methanolic extract of *Bombax ceiba*. *Redox Report* 14 (2009) 41-46.
[19] Zahan, R.; Nahar, L.; Haque, M.; Nesaand, M.L.; Alam Z. Antioxidant and antidiabetic activities of *Alangium salvifolium* and *Bombax ceiba. J. Pharm. Sci.* 12 (2013) 159-163.