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Research Article

Phytochemical investigation and study on biological activities of flowers of *Bombax ceiba*

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Abstract

Phytochemical investigations on the flowers of *Bombax ceiba L.* resulted in isolation and characterization of three compounds scopoletin, stigmastreol and palmitic acid. n-Hexane, chloroform, DCM, ethyl acid, aqueous and methanolic extract of *B. ceiba* flowers were made for screening different bioactivity such as cytotoxicity assay, antimicrobial screening, antioxidant capacity and activity screening, brine shrimp lethality bioassay. n-Hexane extract contained highest percentage of caprylic acid as both free and bound fatty acid. n-Hexane fraction showed low antibacterial activity against *S. aureus* and V. mimicus with zone of inhibition ranging from 0-10 mm. No fraction showed cytotoxicity against HeLa or vero cell line. In brine shrimp lethality bioassay, n-hexane and ethyl acetate extract showed strong cytotoxic potential than the positive control having LC_{50} values 2.91µg/mL and 5.07 µg/mL respectively. Lowest antioxidant capacity was given by the methanolic extract of B. ceiba flower having total antioxidant capacity 23.48 mg/g whereas this fraction showed moderate DPPH scavenging activity (IC_{50}=155.18 µg/mL), highest total antioxidant capacity (69.75 mg/g) was shown by DCM fraction. The highest free radical scavenging was showed by DCM extract (IC_{50}=36.61 µg/mL) and the lowest highest free radical scavenging was showed by hexane extract (IC_{50}=250.63 µg/mL).

Keywords: Phytochemical, *Bombax ceiba* L., cytotoxicity assay, antimicrobial screening, antioxidant capacity, antioxidant activity screening, brine shrimp lethality bioassay.

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1. Introduction

The plant *Bombax ceiba Linn.,*which belongs to Bombacacea family has been investigated for phytochemicals and biological activities. Bombacaceae family consists of about 26 genera and nearly 140 species [2]. *Bombax* (60 species), *Durio* (15species),*Ceiba* (15 species), *Salmalia* (10 species) and *Adansonia* (10species) are some largest genera in this family [3]. *Bombax ceiba L.*, locally known as Shemul, Pagun, cotton tree, Purani, Roktosimul, red-cotton tree, silk cotton tree, Indian kapok [4, 5] etc. It is widely cultivated in Bangladesh, Pakistan, India, China, Vietnam, Ceylon and Malaya [6]. All parts of *Bombax ceiba L.* plant have medicinal values [7]. It is traditionally used as medicinal plant amongst the tribal and local people for treating various diseases of both human and animal [8]. The plant is traditionally used in treating diarrhea, inflammation, catarrhal affection, hypertension, ulceration of bladder and kidney, algesia, hepatotoxicity [9]. Literature survey showed the presence of free β-sitosterol, β-D-glucoside of β-sitosterol, kaempferol, hentriacontane, hentriacontanol and quercetin in the flowers of *B. ceiba*[10]. Isolation of neolignans, phenols, steroids, sesquiterpenoids, and naphthoquinones have been also reported [11]. The present study was performed to isolate and characterize different secondary metabolites and to evaluate potential bioactivity of the flowers of *Bombax ceiba.* *L.*

2. Material and Methods

2.1 Plant Material

Flowers of *Bombax ceiba L.* were collected from Shahbagh, Dhaka, Bangladesh. Then the flowers were taxonomically identified, washed, dried, grinded and preserved in an air-tight container.

2.2 Extraction

The dried powder flowers were extracted exhaustively by cold extraction process using methanol. Dried powder of *Bombax ceiba* flowers (~550g) was soaked in methanol for seven days and filtered and then filtrate was evaporated to dry mass using rotary evaporator. This process was repeated for three times.

2.3 Phytochemical investigation

For separation of extracted compounds into individual pure ones Column Chromatography (CC), Thin-layer Chromatography (TLC) and preparative TLC (PTLC) were used. Ethyl acetate extract of *B. Ceiba* flower (~2.54g) was subjected to column chromatography. For choosing the appropriate solvent mixture in column chromatography, trial method of TLC was employed. Eight different fractions were collected from column (C_1-C_8). Shimadzu FT-IR 4800S Spectrometer was used to characterize the functional group. UV-visible spectrum, ^{1}H-NMR and ^{13}C-NMR and 2D-NMR spectrums were taken for building complete structure of individual compounds.

2.4 Biological investigation

The microbial activity of Hexane, chloroform, dichloromethane, EtOH, aqueous and MeOH extracts of flowers of *Bombax ceiba* were evaluated using agar disc diffusion method. Cytotoxicity test for the different extracts of flowers of *Bombax ceiba* were tested against Hela cell line (a human cervical carcinoma cell) and vero cell line (a kidney epithelial cells extracted from an African green monkey). Brine shrimp lethality assay was investigated by determining the ability of different extracts of *B. Ceiba* to kill laboratory cultured larvae (nauplii). The total antioxidant capacity of the sample (*B.ceiba*) extracts were determined by the Phospho-molybdenum assay method[12] and the DPPH radical-scavenging activity was calculated using the modified method described by Gupta[13].

3. Result and Discussion

Table 1: ^{1}H NMR, ^{13}C NMR data, DEPT, COSY, HSQC and long-range HMBC for compound BC-1

Position No.	Chemical Shift, δ(ppm)				DEPT 135	COSY ^{1}H–^{1}H	HSQC ^{1}H–^{13}C	HMBC ^{1}H–^{13}C	
	δ_H (Mult, J in Hz)		δ_C					^{2}J	^{3}J
	Exp	Rep.[15]	Exp.	Rep. [15]					
O-Me	3.910(s)	3.80	56.40	56.73	+		O-Me	C-6	
2	-		161.80	161.33					
3	6.214(d, 9.6)	6.16 (d)	113.00	113.30	+	7.574	C-3	C-2	C-9
4	7.574(d,9.6)	7.82 (d)	143.63	144.67	+	6.214	C-4		C-2, C-5,C-10
5	6.818(s)	7.17 (s)	107.73	109.98	+		C-5		C-4
6	-		144.43	145.98					
7	-		150.22	151.14					
8	6.865(s)	6.78 (8)	103.26	103.73	+		C-8	C-10	C-9
9	-		111.40	112.09					
10	-		150.09	151.86					

The FT-IR spectrum of compound BC-1 **(Figure 1)** gave peaks at 3337.96, 2947.36, 1704.18, 1608.70, 1436.07, 1566.27, 1509.36, 1291.40, 1263.43, 861.25, 745.52 cm^{-1} which coincided with the reported values [16]. The ^{1}H-NMR of compound BC-1 displayed the aromatic proton signals at 6.214 (1H, d, J=9.6Hz) and 6.818(s), 6. 865 (s) and 7.574 (1H, d, J=9.6). The other signals from BC-1 at δ 1.217, 2.211(q), 3.910(s). In the ^{13}C-NMR tendifferent peaks were observed. Presence of O-methoxy (-OCH_3) observed at δ 56.40, carbonyl group (C=O) observed at δ 161.80ppm. Five non-oxygenated aromatic carbons at δ 113.00, 143.53, 107.73, 103.26, 111.40. Three oxygenated aromatic carbon at δ 144.43, 150.22 and 150.09ppm. DEPT 135 experiment gave six positive peaks at δ 56.39, 77.24, 103.24, 107.70, 113.06, 143.55 ppm. No negative peak was found. Only methyl (-CH_3) or methine (-CH) groups could be present, methylene(-CH_2) group was absent. COSY experiment showed that proton at δ 6.214 is linked with proton at δ 7.574 and proton. HSQC experiment showed that protons at δ 3.910, 6.818, 6.865, 6.214, 7.574 are directly linked with carbon at δ 56.40, 107.73, 103.26, 113.00, 143.55. Based on the physical characteristics and spectral analysis (^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HSQC, HMBC, UV-visible and FT-IR) data of the compound BC-1 and comparing the reported value scopoletin [15,16] the structure of the compound BC-1 **(Figure :1)** was established as scopoletin.

Figure 1: Structure of BC-1 (Scopoletin)

The compound BC-2 **(Figure 2)** was a colorless crystalline solid having R_f value: 0.67 (in 100 DCM) and its melting point was found to be 158-160°C.

Table 2: ^{1}H-NMR, ^{13}C-NMR data for compound BC-2

Position No.	Chemical Shift, δ (ppm)				Position No.	Chemical Shift, δ (ppm)			
	δ_H		δ_C			δ_H		δ_C	
	Exp.	Rep.[17]	Exp.	Rep. [17]		Exp.	Rep.	Exp.	Rep.
1			35.90	37.3	**16**			28.27	29.7
2			28.27	31.7	**17**			56.08	56.1
3	3.50 (m)	3.51 (m)	71.84	71.8	**18**	0.66 (s)	0.69 (s)	12.08	12.1
4			42.24	42.4	**19**	1.01 (s)	1.00 (s)	20.23	19.4
5			140.79	141.0	**20**			39.71	40.0
6	5.346 (br,s)	5.34 (br,s)	121.75	121.7	**21**			21.11	21.1
7			31.49	31.9	**22**	5.00 (dd)	5.04 (dd)	138.34	138.0
8			30.30	31.9	**23**	5.33 (dd)	5.14 (dd)	129.30	128.5
9			50.16	50.2	**24**			51.26	51.0
10			37.28	36.2	**25**			33.74	31.9
11			24.33	21.1	**26**	0.899(d)	0.84 (d)	19.03	19.0
12			37.87	39.5	**27**	0.831(d)	0.80 (d)	21.24	21.3
13			40.51	42.4	**28**			26.10	25.0
14			55.98	56.9	**29**			12.00	12.0
15			25.43	23.2					

The ^{1}H-NMR (400 MHz, in $CDCl_3$) spectrum of compound BC-2 displayed two sharp singlets at δ 0.66 and 1.01 due to presence of methyl proton at C-18 and C-19. The spectrum had a multiplet (triplet of a double doublet, tdd) at δ 3.5 ppm indicative of the presence of oxy-methine proton (C-3)) and H-6 olefinic proton showed a multiplet at δ 5.346. The olefinic protons C-22 and C-23 appeared as characteristics downfield signal at δ 5.00 and 5.334 respectively in proton NMR. Other signals from δ 0.66 to 1.98 is due to presence of different methylene (-CH_2-) and methine (>CH-) protons. In the ^{13}C-NMR (100 MHz, in $CDCl_3$) spectrum showed presence of 29 carbon. Carbons at δ 140.79, 121.75, 138.34, 129.30 are for olefinic carbons at C-5, C-6, C-22 and C-23 respectively. Carbons at δ 40.51 and 37.28ppm are for quaternary carbon at C-13 and C-10. Methyl carbons gave signals at 21.24 (C-27), 21.11 (C-21), 19.03 (C-26), 20.23 (C-19), 12.08 (C-18) and 12.00 (C-29) ppm. Signal 71.85 was for >CH-OH carbon at C-3.42.24 (C-4), 37.87 (C-12), 35.91 (C-1), 31.49 (C-7), 28.27 (C-2), 28.27 (C-16), 26.10 (C-28), 25.43 (C-15) and 24.33(C-11) are for methylene carbons. Methine carbons gave signal at 55.98 (C-14), 56.09 (C-17) 51.26 (C-24), 50.16 (C-9), 39.71 (C-20), 33.74 (C-25) and 30.30 (C-8) ppm. Comparing the reported value [17] of ^{1}H-NMR and ^{13}C-NMR spectral data compound BC-2 **(Figure 2)** is stigmasterol.

Figure 2: Structure of BC-2 (Stigmasterol)

The compound BC- 3 **(Figure: 3)** was a white solid having R_f value: 0.73 (in 100 DCM) and its melting point was found to be 61-63° C.

Table 3: ^{1}H-NMR, ^{13}C-NMR data for compound BC-3

^{1}H NMR			^{13}C NMR		
Proton No.	**δ_H (ppm)**		**Carbon No.**	**δ_C (ppm)**	
	Experimental	**Reported Value**		**Experimental**	**Reported Value**
1	-	11.0	**1**	179.12	178.8
2	2.316(t)	2.30(t)	**2**	33.89	34.0
3	1.99(m)	1.52	**3**	24.70	24.7
4	1.62(m)	1.29	**4-13**	28.93-29.77	29.0-29.6
5-15	1.30 (m)	1.31(m)	**14**	31.93	31.9
16	0.868(t)	0.88(t)	**15**	22.70	22.7
			16	14.13	14.1

The ^{1}H-NMR (400 MHz, in $CDCl_3$) spectrum of compound BC-3 displayed four type of proton signals at δ 0.868 (t), 1.30(m), 1.62(m), 1.99(m) 2.335(t) and 7.250 ($CDCl_3$).

In the ^{13}C-NMR (100 MHz, in $CDCl_3$) spectrum seven types of different peaks were observed. Presence of carboxylic acid group (C-1) observed at δ 179.12. Other six types of carbon peaks are at δ 14.13 (C-17), 22.70(C-16), 24.7 (C-3), 28.93-29.77 (C-4~C-14), 31.93 (C-15), 33.89 (C-2)

Based on the physical characteristics and spectral analysis (^{1}H-NMR and ^{13}C-NMR) data of the compound BC-3 and comparing the reported value of ^{1}H-NMR and ^{13}C-NMR spectral data of palmitic acid, the structure of the compound BC-3 was established as palmitic acid having the following structure:

Figure 3: Structure of BC-3 (Palmitic acid)

3.1 Biological investigation:

3.1.1 Antimicrobial screening:

In this investigation, five gram positive bacteria (*Bacillus cereus,Bacilliussubtilis, Bacillus megateriam, Staphylococcus aureus, Sarcinalutea*), eight gram negative bacteria (*Escherichia coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas aureus, Vibrio mimicus, Shigella dysenteriae, Vibrio parahaemolyticus, Shigellaboydii*) and three fungi (*Saccharomyces cerevisiae, Candida albicans, Aspergillus niger*) were used. Ciprofloxacin (5µg/disc) standard disc was used as the reference. n-Hexane partitionate showed low antibacterial activity against *Staphylococcus aureus* and *Vibrio mimicus* with zone of inhibition ranging from 0-10 at the constant sample concentration 400 µg/disc**.**

3.1.2 Brine Shrimp lethality bioassay:

Vincristine sulfate (VS) was used as positive control and the LC_{50} was found 9.73 µg/mL for VS. HF extract revealed significant lethality whereas DCMF, EA and AF extractives revealed moderate activity compared to this positive control. CF and MF fractions showed very low activity.

Table 5: LC_{50} values of the test samples of *B. ceiba* flower

Test Samples	**VS**	**HF**	**CF**	**DCMF**	**EAF**	**AF**	**MF**
Regression line	y =31.994x+ 18.404	y =27.344x +37.304	y =31.002x +16.663	y =25.421x +14.744	y =26.601x +31.247	y = 31.25x +22.348	y = 34.35x +13.414
R^2	0.9740	0.9328	0.9747	0.9319	0.9349	0.9733	0.9506
LC_{50}(µg/mL)	9.73	2.91	11.88	24.32	5.07	7.67	11.61

3.1.3 Antioxidant activity:

Among all extractives of *B. ceiba* the highest free radical scavenging was showed by (36.61 µg/mL) and the lowest highest free radical scavenging was showed by DCM extract hexane extract (250.13 µg/mL). Ascorbic acid was used as standard.

Table 6: IC_{50} values of the standard and different fractions of the flowers of *Bombax ceiba*

Test Sample	**HF**	**DCMF**	**MF**	**Ascorbic acid**
IC_{50} (µg/mL)	250.63	36.61	155.18	2.16

3.1.4 Antioxidant capacity:

The total antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry extract using the equation obtained from a standard ascorbic acid calibration curve, y = 0.0083x-0.0149, R^2= 0.9945

Table 7: Total antioxidant capacity of different extract of *B. ceiba*

Test Sample	HF	CF	DCMF	EAF	AF	MF
Total antioxidant capacity (mg/g)	25.29	50.95	69.75	38.90	28.06	23.48

4. Conclusion

A detailed phytochemical analysis has been carried out on the flowers of *Bombax ceiba L.* During this investigation five compounds were isolated from this flowers extract. Among them complete structure of three compounds were established including Scopoletin, Stigmasterol and Palmitic acid. Structures of these isolated compound were established using different spectroscopic method (^{1}H NMR, ^{13}C NMR, FT-IR and UV-visible spectroscopy). Fourth crystalline compound was a tertiary aromatic amide having hydroxyl group determined by FT-IR spectroscopy. Bioactivity of different fractions (n-hexane, chloroform, DCM, ethyl acetate, aqueous, methanolic fractions) were investigate such as antioxidant activity, antioxidant capacity, antimicrobial screening, cytotoxicity assay using cancer and non-cancer cell line and by using brine shrimp lethality assay. The species showed significant antioxidant activity, brine shrimp lethality and low antimicrobial activity for a constant sample concentration but showed no cytotoxic effect on cancer cell line. Considering the potential bioactivity and presence of different isolable secondary metabolites, the plant parts should be studied further by using more sophisticated methods to find out unexplored efficacy and to help in the discovery of new drugs.

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Conflicts of Interest

The authors confirm no conflicts of interest.

References

[1]. Mir, M.A., et al., *Antidiabetic properties and metal analysis of Bombax ceiba flower extracts. Global J. Addict. Rehabil. Med*, 2017; 1(3): 1-7.

[2]. Chaudhary, P.H. and S.S. Khadabadi, Bombax ceiba Linn.: pharmacognosy, ethnobotany and phyto-pharmacology. *Pharmacognosy Communications*, 2012; **2**(3): 2-9.

[3]. Mub, B., *A brief introduction to Semal (Bombax ceiba Linn).* Available at h ttp://www. f orestrynepal. org/images/simal present report3. pdf, 2007.

[4]. Saleem, R., et al., Hypotensive, hypoglycaemic and toxicological studies on the flavonol C-glycoside shamimin from *Bombax ceiba. Planta medica*, 1999. 65(04): p. 331-334.

[5]. Chakraborty, D., V. Ravi, and P. Chakraborty, Phytochemical evaluation and TLC protocol of various extracts of *Bombax ceiba* Linn. *International Journal of Pharmaceutical Sciences and Research*, 2010; 1(8): p. 66-73.

[6]. You, Y.J., et al., Antiangiogenic activity of lupeol from *Bombax ceiba. Phytotherapy Research* 2003; 17(4): p. 341-344.

[7]. Said, A., et al., Phytoconstituents and bioctivity evaluation of *Bombax ceiba* L. flowers. *Journal of Traditional Medicines*, 2011; 28(2): 55-62.

[8]. Jain, V., et al., Free radical scavenging property of *Bombax ceiba* Linn. root. *Res J Med Plant*, 2011; 5(4): 462-470.

[9]. Patel, S.S., et al., Cardioprotective effect of Bombax ceiba flowers against acute adriamycin-induced myocardial infarction in rats. *Revista Brasileira de Farmacognosia,* 2011; 21(4): 704-709.

[10]. Rameshwar, V., et al., A Pharmacognostic and pharmacological overview on *Bombax ceiba.* Scholars *Academic Journal of Pharmacy*, 2014; 3(2): 100-107.

[11]. Tundis, R., et al., *In vitro* cancer cell growth inhibition and antioxidant activity of Bombax ceiba (Bombacaceae) flower extracts. *Natural product communications*, 2014; **9**(5): 1934578X1400900527.

[12]. Gioda, A. and M.d.R. Prieto, Histoire des sécheresses Andines; Potosi, El Niño et le petit âge glaciaire. *La Météorologie*, 1999.

[13]. Marinova, G. and V. Batchvarov, Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian Journal of Agricultural Science*, 2011; 17(1): 11-24.

[14]. Park, P. and R. Goins, In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science*, 1994; 59(6): 1262-1266.

[15]. Prabowo, W.C., K.R. Wirasutisna, and M. Insanu, Isolation and characterization of 3-acetyl aleuritolic acid and scopoletin from stem bark of *Aleurites moluccana* (L.) Willd. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 5: 851-853.

[16]. Bhatt Mehul, K., K. Dholwani Kishor, and K. Saluja Ajay, Isolation and structure elucidation of scopoletin from *Ipomoea reniformis (Convolvulaceae). Journal of Applied Pharmaceutical Science,* 2011; 1(05): 138-144.