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A Comparative Phytochemical and Biological Study between Different Solvent Extracts of *Bombax ceiba* Roots Available in Bangladesh

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Abstract

Use of different solvent systems for extraction of plant materials may cause variation in their bioactivities. The present study was conducted to evaluate the presence of different phytoconstituents and to compare *in vitro* bioactivities of petroleum ether, dichloromethane (DCM) and methanol extracts of *Bombax ceiba* (*B. ceiba*) roots available in Bangladesh. Preliminary phytochemical screening was conducted using specific standard procedure. Antioxidant activity of the extracts was evaluated using DPPH radical scavenging assay. Determination of total phenolic and flavonoid content was also carried out. Antibacterial and cytotoxic activities were investigated using disc diffusion method and brine shrimp lethality bioassay, respectively. All the experiments were carried out from February 2016 to September 2016. Phytochemical evaluation revealed the presence of alkaloids, terpenoids, carbohydrates, tannins, flavonoids, saponins and steroids. The methanol extract showed the highest DPPH radical scavenging activity and had the highest phenolic (187.42 ± 3.77 mg/g, GAE) and flavonoid content (74.67 ± 4 mg/g, QE) followed by the DCM and petroleum ether extracts. The extracts showed positive correlation between DPPH radical scavenging activity with the phenolic and flavonoid content. All the extracts showed mild to moderate *in vitro* antibacterial activity with zone of inhibition ranging from 7 mm to 13 mm. In brine shrimp lethality bioassay, the observed LC_{50} values for petroleum ether, DCM and methanol extracts were 70.72 µg/ml, 37.72 µg/ml and 22.58 µg/ml, respectively which revealed strong cytotoxic potential of the extracts compared to the positive control. The results indicated that *B. ceiba* roots could be a very potent source of natural radical scavenger and cytotoxic agent.

Keywords

Bombax ceiba, Antioxidant Activity, DPPH, Antibacterial Activity, Cytotoxic Effect

1. Introduction

Bombax ceiba (Local name: Silk Cotton tree, Family: Bombacaceae), is widely distributed in Bangladesh and also in temperate Asia, tropical Asia, western Africa and Australia [1]. For years, this plant has been used by the tribal populations of Asia. Stem barks of *B. ceiba* is used to cure gonorrhea, impotency, spermatorrhea, nocturnal emission and leucorrhoea by the people of Orissa, India. In Bangladesh, seeds and roots of *B. ceiba* have been used traditionally for the treatment of leprosy. Roots and barks of *B. ceiba* have been used to treat muscular injury by Chinese population [2]. Barks of *B. ceiba* have been found to lower blood glucose level, triglyceride level as well as cholesterol level in streptozotocin induced diabetic rats [3]. Studies also suggested that the stem, flowers and leaves of *B. ceiba* possess hepatoprotective, hypotensive, antioxidant properties as well as strong anti-inflammatory, antiviral, antibacterial and analgesic activities [4] [5]. It has been claimed in Ayurveda that the roots of *B. ceiba* are stimulant, astringent, restorative, demulcent, emetic and tonic [6].

Free radicals are crucial to any biochemical process and in case of aerobic life they play an important role in body's metabolism process. During respiration and some cell mediated immune functions, free radicals are continuously produced. Heart disease, cancer, diabetes, rheumatic disorder as well as aging are associated with excess level of free radicals [7]. Antioxidants, on the other hand, are the agents that react with the free radicals and neutralize their effects. Thus, antioxidants help to prevent many disorders that are associated with high level of oxidative stress. *B. ceiba* is one of the natural sources that have been reported to show antioxidant property [8].

It was found that reactive oxygen species (ROS) can change cell's normal gene transcription and signal transduction process followed by damage to DNA by modifying the base and altering DNA strands [9]. So it is very essential to find a natural agent which can be used safely as antioxidants as well as cytotoxic agents [10].

Multi-drug resistant microbial strains are increasing day by day and they show reduced susceptibility to antibiotics. The indiscriminate use of broad-spectrum antibiotics and immunosuppressive agents contribute to the multi-drug resistance and also various side effects [11]. Researchers are giving priority to identify new antimicrobial agents from nature. It has been observed that the secondary metabolites of higher plant origin may show novel mechanism of action along with their antimicrobial activity [12]. Besides, solvents with different polarity have pronounced effect on extracting bioactive molecules which cause variation in their bioactivities [13]. So, as a part of our ongoing investigation on local me-

dicinal plants of Bangladesh and an attempt to evaluate the variation in pharmacological activities due to different solvent system [14] [15], we have reported here a study on the antioxidant, antimicrobial and cytotoxic activities of the different solvent extracts of *B. ceiba* roots.

2. Materials and Methods

2.1. Chemicals and Solvents

Chemicals and reagents were obtained from Sigma-Aldrich co. (USA), Merck (Germany) and Fine Chemicals (India). All the solvents used throughout the experiments were of analytical grades.

2.2. Plant Collection and Extraction of the Plant Material

The plant material was collected in February, 2016 from Rampura, Dhaka, Bangladesh and was identified and authenticated by Mr. Abdur Rahim, Technical Officer, Department of Botany, Jahangirnagar University. The roots were dried and pulverized into coarse powder. Two hundred grams of powdered plant materials were taken into sealed container and macerated for seven days at room temperature using 1.0 liter of petroleum ether with occasional shaking and stirring. Same procedure was repeated using dichloromethane (DCM) and methanol as solvents. The extracts were then filtered using sterilized cotton filter followed by whatman no 1 filter papers. The solvents were totally evaporated using rotary evaporator under reduced pressure at 40˚C - 50˚C temperature. The obtained crude extracts were collected, weighed and then stored at 4˚C until further use. Table 1 shows the yield percentages (w/w) of *B. ceiba* roots in different solvents used for extraction.

2.3. Phytochemical Screening

Chemical tests were carried out on the freshly prepared extracts using standard procedures to assess the existence of active phytochemical constituents. The following reagents and chemicals were used: alkaloids with Wagner's and Hager's reagent, carbohydrates with Molisch's test, tannins with 0.1% ferric chloride, terpenoids with modified Salkowski test, flavonoids with the use of concentrated hydrochloric acid, saponins with distilled water and glycosides by using alcohol with few drops of H_2SO_4, followed by neutralization with NaOH solution and boiling with Fehling's solution. These were identified by characteristic color changes using standard procedures [16].

Table 1. Yield % of *B. ceiba* roots in different solvent extracts.

Solvent used	Yield %
Petroleum ether	1.2
Dichloromethane	3.4
Methanol	3.8

2.4. Quantitative Phytochemical Analysis

2.4.1. Determination of Total Phenolic Content

To determine the total phenolic content, 1 ml of each extract (250 µg/ml) and the standard gallic acid (50 - 250 µg/ml) were oxidized with 10% Folin-Ciocalteu reagent and then neutralized with 700 mM sodium carbonate solution. The obtained mixtures were allowed to stand for 60 minutes at room temperature and absorbance of the resulting solutions was taken at 765 nm against reagent blank [17]. A standard curve was prepared for gallic acid (**Figure 1**) and used to determine the total phenolic content of the extracts. The results were expressed as gallic acid equivalents (mg/g, GAE) and as mean ± SD.

2.4.2. Determination of Total Flavonoid Content

Aluminium chloride colorimetric method [18] was used to determine the flavonoid content. One milliliter of each extract (250 µg/ml) in methanol was mixed with 200 µl of 10% w/v aluminium chloride and 1 M potassium acetate solution. Then 5.6 ml distilled water was added to the mixture and was kept at room temperature for 40 minutes. The absorbance of the resulting solutions was measured at 415 nm against reagent blank. Total flavonoid content of the extracts was determined from a standard curve prepared for quercetin (50 - 250 µg/ml) solution (**Figure 2**). The estimated results were expressed as quercetin equivalents (mg/g, QE).

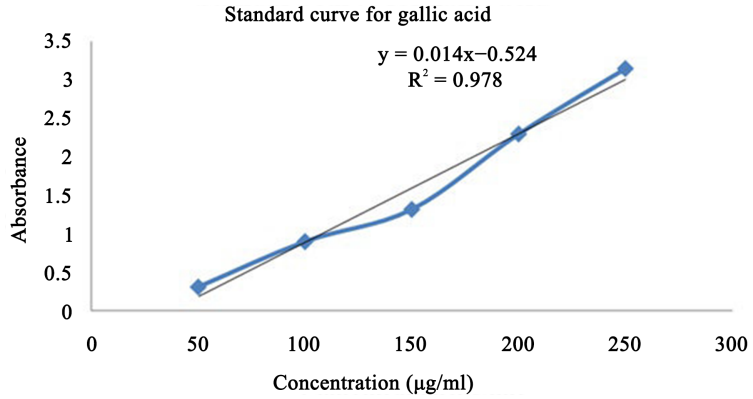


Figure 1. Standard curve for gallic acid.

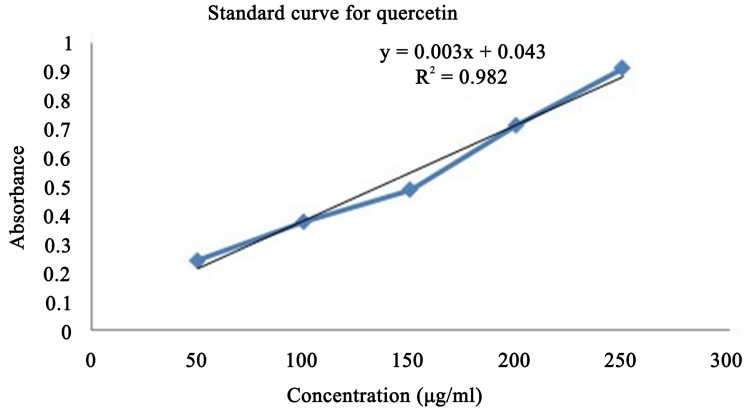


Figure 2. Standard curve for quercetin.

2.5. Antioxidant Activity

DPPH Radical Scavenging Activity

Methanol was used to prepare a stock solution (400 µg/ml) of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 100 µl of this stock solution was added to 5 ml solution of the extracts and the standard (ascorbic acid) of different concentrations (12.5 - 200 µg/ml). After proper mixing the solutions were kept in dark for 20 minutes and by using a spectrophotometer (Shimadzu UV PC-1800), the absorbance of the solutions were measured at 517 nm against methanol. Scavenging activity was measured by reduction in the absorbance of methanol solution of DPPH and expressed as the percentage inhibition using the formula:

$$\left[\left(A_0 - A_1\right)/A_0\right]\times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. IC_{50} values were calculated from the graph constructed as a plot of % inhibition vs. concentration [19].

2.6. Antibacterial Assay

Five Gram-positive and six Gram-negative bacterial strains were used in the antibacterial assay following the disc diffusion method [20]. Suspension of each microorganism (100 µl, containing approximately 100 - 150 CFU/ml) was spread over nutrient agar media and dried, sterile filter paper discs of 6 mm diameter were placed gently on those agar plates after impregnated with 300 µg and 600 µg of different extracts prepared by dissolving them in methanol followed by evaporation. After 24 hours of incubation at 37˚C the diameter of zone of inhibition was measured in mm. Kanamycin (30 µg/disc) was used as positive control while blank disc (impregnated with solvents) was used as negative control.

2.7. Cytotoxic Activity

Cytotoxicity of different concentrations of each extract was identified using the brine shrimp lethality bioassay [21]. The eggs of brine shrimp (*Artemia salina* Leach) were hatched in a tank containing artificial sea water for 48 hours at a temperature around 37˚C and pH 8.4 with constant oxygen supply and allowed to be matured as nauplii. The extracts were dissolved in DMSO (0.5% v/v) and diluted to get final concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/ml. Seawater was added to the marked test tubes containing extracts to make final volume 5 ml and 10 living nauplii were taken into each of the test tube. By keeping the total volume same, DMSO was taken as negative control and solutions of different concentrations of vincristine sulphate were taken as positive control. The number of surviving nauplii in the test tubes was counted after 24 h and the percentage of lethality was calculated for each concentration of the extracts and standard.

2.8. Statistical Analysis

Statistical analyses were performed using Microsoft Excel, 2007. All experiments were repeated three times and results were expressed as mean values ± SD.

3. Results

3.1. Phytochemical Screening

The phytochemical analysis of all extracts of *B. ceiba* roots revealed the presence of alkaloids, terpenoids, carbohydrates, tannins, flavonoids, saponins and steroids in varying amounts (Table 2).

3.2. Quantitative Phytochemical Analysis

3.2.1. Total Phenolic Content

Total phenolic content of the extracts was calculated using the standard curve plotted for gallic acid ($y = 0.014x - 0.524$; $R^2 = 0.978$) (Figure 1). The methanol extract showed the highest amount of phenolic compounds (187.42 ± 3.77 mg/g, GAE) followed by the DCM (176.28 ± 1.59 mg/g, GAE) and petroleum ether extracts (162.47 ± 4.52 mg/g, GAE) (Table 3).

3.2.2. Total Flavonoid Content

Total flavonoid content of the extracts was calculated using the standard curve plotted for quercetin ($y = 0.003x + 0.043$; $R^2 = 0.982$) (Figure 2). The highest amount of flavonoid content was present in methanol extract (74.67 ± 4 mg/g, QE) whereas the petroleum ether extract contained the lowest amount of flavonoid content (43.55 ± 2.77 mg/g, QE) (Table 3).

Table 2. Results of chemical group tests of the extracts of *B. ceiba* roots.

Plant extract	Alkaloids	Terpenoids	Carbohydrates	Tannins	Flavonoids	Saponins	Steroids
Petroleum ether	+	+	+	+	+	+	+
DCM	+	++	+	+	+++	+	+
Methanol	++	++	+	++	++	++	++

+++: highly present, ++: moderately present, +: slightly present. DCM: Dichloromethane.

Table 3. Total phenolic content and total flavonoid content of different extracts of *B. ceiba* roots.

Extracts	Total phenolic content (in mg/g, GAE)	Total flavonoid content (in mg/g, QE)
Petroleum ether	162.47 ± 4.52	43.55 ± 2.77
DCM	176.28 ± 1.59	64.89 ± 3.35
Methanol	187.42 ± 3.77	74.67 ± 4.0

Values are represented as mean ± SD (n = 3). DCM: Dichloromethane.

3.3. Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

From the analysis of Figure 3, it can be concluded that the scavenging effect of the extracts was increased in a concentration dependent manner. The highest radical scavenging activity was shown by methanol extract (IC_{50}: 144.77 ± 3.61 µg/ml) whereas petroleum ether extract showed the lowest activity (IC_{50}: 214.83 ± 5.37 µg/ml). The IC_{50} values for the extracts and the standard (ascorbic acid) are given in Table 4.

3.3.2. Correlation between Total Phenolic/Flavonoid Content with DPPH Scavenging Activity

Correlation curves of DPPH scavenging assay with total phenol and flavonoid content had been developed (Figure 4(a) and Figure 4(b)). The Pearson's correlation coefficient (r) and coefficient of determination (R^2) (r = 0.996, R^2 = 0.994) between total flavonoid content and DPPH scavenging activity was higher than those of total phenol content and DPPH scavenging activity (r = 0.974, R^2 = 0.949).

3.4. Antibacterial Assay

When compared to the standard disc of kanamycin (30 µg/disc), the DCM extract showed mild to moderate antibacterial activities against all the strains except *Salmonella typhi* and the highest activity was observed against *Sarcina lutea*

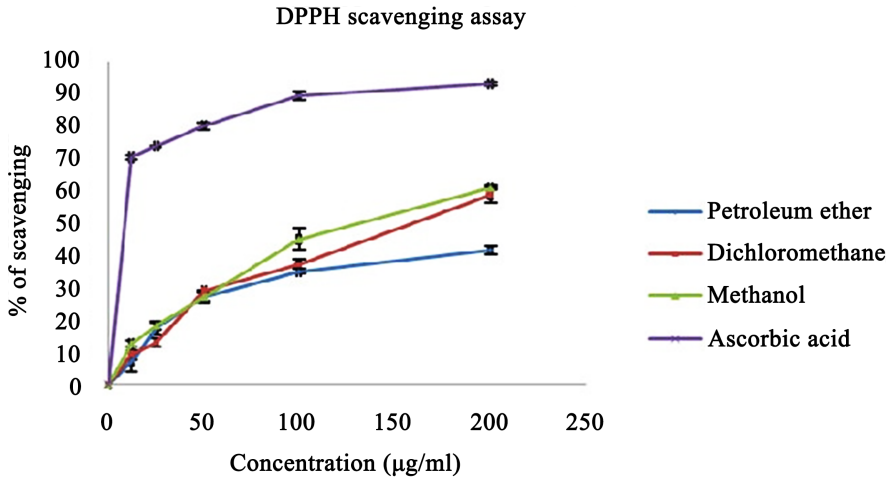
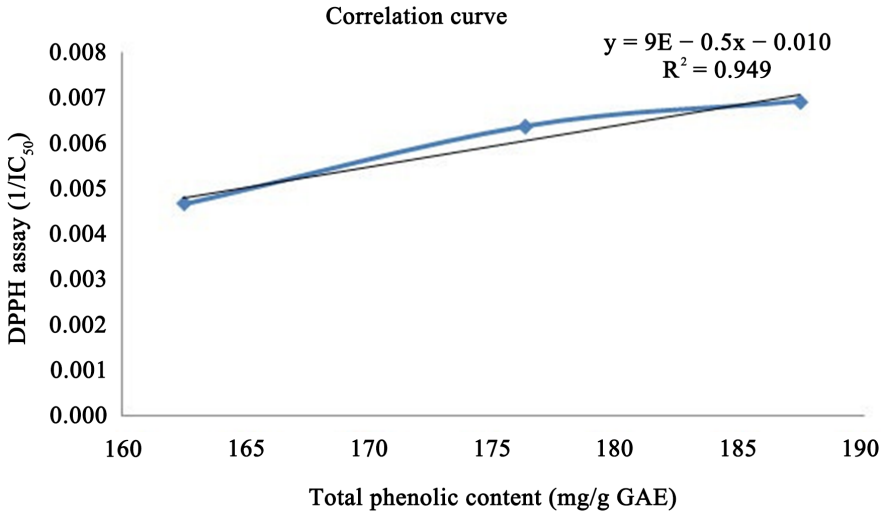


Figure 3. DPPH scavenging activity of different extracts of *B. ceiba* roots and ascorbic acid.

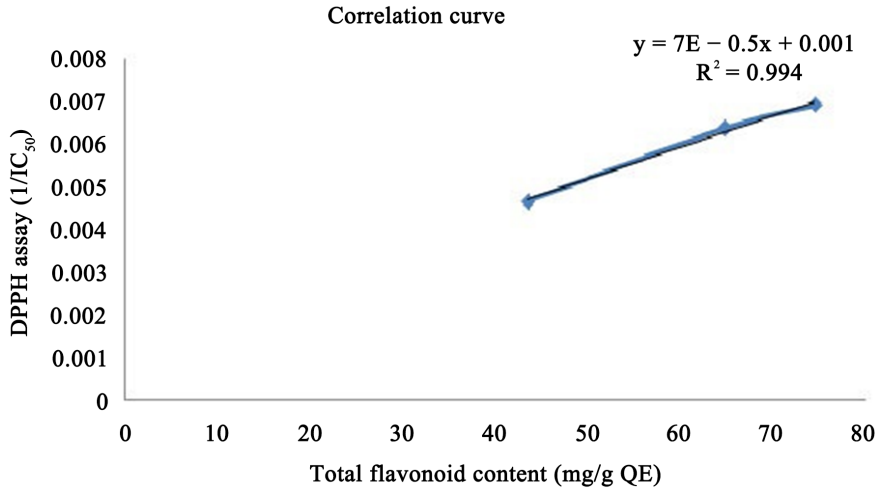
Table 4. IC_{50} values of different extracts of *B. ceiba* roots and ascorbic acid.

Extract/standard	IC_{50} values (µg/ml)
Petroleum ether	214.83 ± 5.37
DCM	157.23 ± 5.39
Methanol	144.77 ± 3.61
Ascorbic acid	2.02 ± 0.24

DCM: Dichloromethane.



(a)



(b)

Figure 4. (a) Correlation curve between DPPH scavenging assay and total phenolic content; (b) Correlation curve between DPPH scavenging assay and total flavonoid content.

(12.3 ± 1.5 mm at 600 µg/disc). The methanol extract displayed the highest zone of inhibition against *Escherichia coli* (10.3 ± 1.5 mm at 600 µg/disc) although it did not show antibacterial activity against some of the strains. The petroleum ether extract was found active only against *Staphylococcus aureus* and *Escherichia coli* (Table 5).

3.5. Cytotoxic Activity

In brine shrimp lethality bioassay, the lowest LC_{50} value (22.58 µg/ml) was calculated for the methanol extract and the highest LC_{50} value (70.72 µg/ml) was estimated for the petroleum ether extract. The extracts showed strong cytotoxic activity compared to the standard vincristine sulphate (LC_{50}: 1.24 µg/ml) (Table 6 and Table 7).

Table 5. Zone of inhibition of different extracts of *B. ceiba* roots and kanamycin.

Microorganisms	Petroleum ether		DCM		Methanol		Kanamycin
	300 µg/disc	600 µg/disc	300 µg/disc	600 µg/disc	300 µg/disc	600 µg/disc	30 µg/disc
Gram-positive bacteria Zone of inhibition (mm)							
Sarcina lutea	-	-	8.3 ± 0.5	12.3 ± 1.5	-	-	30.1 ± 0.1
Bacillus megaterium	-	-	-	7.3 ± 0.5	-	8.6 ± 0.5	30.0 ± 0.5
Bacillus subtilis ATCC 6059	-	-	8.0 ± 0.1	11.0 ± 0.1	-	-	30.3 ± 0.3
Staphylococcus aureus ATCC 25923	-	7.3 ± 0.5	7.3 ± 0.5	10.1 ± 0.1	-	-	34.0 ± 0.5
Bacillus cereus ATCC 14579	-	-	-	8.3 ± 0.5	-	7.5 ± 0.5	30.0 ± 0.5
Gram-negative bacteria							
Pseudomonas aeruginosa ATCC 27853	-	-	8.4 ± 0.1	11.0 ± 1.0		7.3 ± 0.5	28.1 ± 0.1
Salmonella typhi ATCC 13311	-	-	-	-	-	7.3 ± 0.5	17.5 ± 0.6
Escherichia coli ATCC 25922	-	7.3 ± 0.5	8.0 ± 1.0	12.0 ± 0.5	8.1 ± 0.1	10.3 ± 1.5	30.3 ± 0.3
Vibrio mimicus ATCC 33653	-	-	-	8.0 ± 0.5	-	-	13.5 ± 0.6
Shigella boydii ATCC 13147	-	-	-	8.2 ± 0.2	-	-	23.0 ±0.5
Shigella dysenteriae ATCC 26131	-	-	8.5 ± 0.6	9.6 ± 0.6	7.5 ±0.5	9.3 ± 1.5	24.2 ± 0.2

Values are expressed as mean ± SD; n = 3. '-' indicates no zone of inhibition. DCM: Dichloromethane.

Table 6. % mortality of brine shrimp larvae at different concentrations of extracts and vincristine sulphate.

Concentration (µg/ml)	Log C	% mortality			
		Petroleum ether	DCM	Methanol	Vincristine sulphate
400	2.60206	90	100	100	100
200	2.30103	70	80	100	100
100	2	50	70	80	100
50	1.69897	40	50	70	100
25	1.39794	30	30	40	100
12.5	1.09691	10	20	30	90
6.25	0.79588	10	10	10	90
3.125	0.49485	10	10	10	70
1.5625	0.19382	0	0	0	50

% mortality of brine shrimp larvae after 24 h. DCM: Dichloromethane.

Table 7. Cytotoxic activity of various extracts of *B. ceiba* roots and vincristine sulphate.

Extracts	LC_{50} values (µg/ml)
Petroleum ether	70.72
DCM	37.72
Methanol	22.58
Vincristine sulphate	1.24

DCM: Dichloromethane.

4. Discussion

The medicinal value of plants links to presence of bioactive phytochemicals that display definite physiological action on human which can be used in treating many diseases. Previous studies on different medicinal plants showed that the existence of secondary metabolites are correlated with the therapeutic properties such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic etc. [22]. In this study, phytochemical tests demonstrated the presence of alkaloids, terpenoids, carbohydrates, tannins, flavonoids, saponins and steroids in all extracts in varying amounts.

DPPH radical scavenging activity can be used to measure the electron donating ability and free radical scavenging ability of natural products [23]. In present days, use of natural antioxidants get higher acceptances over synthetic antioxidants because of various toxic effects of the later on human body [24]. In this study, petroleum ether, DCM and methanol extracts of *B. ceiba* roots exhibited moderate to strong concentration dependent DPPH radical scavenging activity compared to the standard (ascorbic acid) which also showed consistent results with the previous studies done by others [5] [25].

Determination of phenolic compounds in plant extract has proven to be significantly important as polyphenolic compounds in plants can act as free radical scavengers or antioxidants. The aromatic (benzene) rings of the polyphenols are substituted by hydroxyl groups or other functional derivatives. These groups have the capability to absorb free radicals and reactive oxygen species (ROS) which may lead to several pathological conditions including cancer [26] [27]. Among the polyphenols, flavonoids also prevent body from various diseases as their molecular structure and position of hydroxyl groups make them potent antioxidants [28]. Besides different types of chemical compounds are dissolved in solvents with different polarity, such as high amount of phenolic compounds are dissolved in polar solvents [29]. Our study exhibited similar results where the methanol extract of *B. ceiba* roots showed the highest amount of phenol and flavonoid content compared to petroleum ether and DCM extracts. Moreover, correlation curves of DPPH scavenging assay with total phenol and flavonoid content had been developed. Literature reports have suggested that there is a correlation between the total phenolic content and antioxidant activity of plant extracts [30]. The high correlations show that the presence of phenol and flavonoid compounds in the extracts may contribute to their antioxidant activity.

DCM and methanol extracts exhibited mild to moderate antibacterial activity against certain Gram-positive and Gram-negative bacteria whereas petroleum ether exhibited very low activity only against *S. aureus* and *E. coli.* Among the three extracts, DCM extract exhibited antibacterial activity against all the Gram-positive and Gram-negative bacteria except *S. typhi* examined at the concentration of 600 µg/disc. The antibacterial activity exerted by the extracts may be due to the likely presence of alkaloids, steroids and tannins [31] [32] [33], although this needs to be confirmed by further research.

Petroleum ether, DCM and methanol extracts of *B. ceiba* roots showed concentration dependent increment in percent mortality of brine shrimp nauplii. In this study, methanol extract showed the highest cytotoxic activity (LC_{50}: 22.58 µg/ml) compared to DCM and petroleum ether extracts. All the extracts showed strong cytotoxic activities compared to the standard vincristine sulphate. Phytochemical screening indicated the likely presence of terpenoids which are reported to have bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anticancer and antiallergic activity [34]. Thus, the observed cytotoxic activity may be attributed to the presence of terpenoids; however, this needs to be confirmed. The potential cytotoxic activity exhibited by the extracts used in this study is also supported by other works that demonstrated strong cytotoxic activity of *B. ceiba* [35] [36].

The results obtained from the study showed that different extracts of *B. ceiba* roots possess potential antioxidant, antibacterial and cytotoxic properties which demonstrate clearly the scientific basis of traditional uses of the plant. So, the present study suggests that *B. ceiba* roots can be a useful source to lead the development of new drugs. Further studies are required to establish its antioxidant activity by other *in vitro* and *in vivo* methods, cytotoxic activity against different cancer cell lines and to identify the chemical compounds responsible for such activities.

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Conflict of Interest Statement

We declare that we have no conflict of interest.

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