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Phytochemical screening and biological studies of the stems of *Polyalthia suberosa* (Roxb.)

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Abstract
Polyalthia suberosa, a long been used medicinal plant was undertaken to investigate for phytochemical screening and biological activities. The phytochemical screening of the stems of *Polyalthia suberosa* upon investigation showed the presence of alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. Biological activities of different extracts of the plant stems (hexane, DCM, methanol and water) were tested using well-established methods. The antimicrobial activity was carried out using disc diffusion method. All the extracts showed more sensitivity against gram-negative bacteria than towards gram-positive bacteria providing significant zone of inhibition. Free radical scavenging activity using DPPH method showed significant free radical scavenging activity with low IC_{50} values for methanol and water extracts of the stems of *Polyalthia suberosa*. Cytotoxic activity tested on *Artemia salina* samples showed good results of mortality for all the extracts but the best result was found for methanol extract. Total antioxidant capacity of the above-mentioned extracts provided strong results for all the extracts, but methanolic extract came out to be the most potent.

Keywords: *Polyalthia suberosa*, phytochemical screening, antimicrobial activity, cytotoxicity, free radical scavenging activity, total antioxidant capacity

Introduction
Plant and plant products are being used as a source of medicine for very long. Medicinal plants are nature's gift to human beings to lead a disease-free, healthy life. They are rich sources of bioactive compounds and thus serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. The plant *Polyalthia suberosa* Roxb. (Locally known as Borochalli), belonging to the family Annonaceae, is a shrub or tree, widely distributed in Bangladesh [1]. The plant has been reported to be used as a bitter tonic, abortifacient, febrifuge and cure of sorption stings all over the world [2].
Polyalthia suberosa grows up to a height of 2-4 meters, leaves are oblong to narrowly oblong-obovate, 5 to 11 centimeters long and flowers are solitary, pale-yellow, about 1 cm long or less [3]. Bark and seed are considered as diuretic, soporific and sedative, plant mucilage has a wide range of applications as a thickening, binding, disintegrating, suspending, emulsifying and gelling agents [4]. Fruits are edible and serve as a good source of nutrients [5].
A large number of pharmacologically active compounds have been isolated from different parts of *Polyalthia suberosa* [6-10]. Alkaloids, flavonoids, sterols, triterpenes etc. important medicinally active compounds have been isolated from stems, leaves and stem bark of *Polyalthia suberosa* These types of compounds show antiviral [6, 7], anti-HIV, antibacterial, analgesic, antidiarrhoeal and antifungal activities against various virus, bacteria, and fungi [10-12].
The present study deals with the investigation of phytochemical screening and biological activities of different extracts of the stems of *Polyalthia suberosa*. The antimicrobial activity, free radical scavenging activity, cytotoxicity and total antioxidant capacity have been reported.

Materials & Methods
Chemicals and solvents
Reagents employed, are all of analytical grade (Merck and BDH) and were distilled before use.

Sample collection
The stems of *Polyalthia suberosa* were collected from Savar, Dhaka. A voucher specimen of this was deposited in the Bangladesh National Herbarium (BNH) having ACCESSION NO DACB 38380. The stems of the plant *Polyalthia suberosa* were dried after collection and then ground to coarse powder using a cyclotec grinding machine.

Extraction of the plant material

The powdered stems of *Polyalthia suberosa* (40g) were extracted with hexane (60-80) ˚C followed by dichloromethane (DCM), methanol and water. All the extracts were separately concentrated to dry mass using a rotary vacuum evaporator at 40 ˚C, under reduced pressure. The dry mass of hexane, DCM, methanol and water extracts were used for biological activity determination.

Phytochemical screening

Chemical tests for a different class of compounds were carried out on the aqueous extract and the powdered specimens using standard procedures to identify the phytochemical constituents as alkaloid, terpenoid, flavonoid, steroid, tannin, saponin and cardiac glycoside [13].

Antimicrobial screening

For antimicrobial screening using disc-diffusion method, all the extracts (hexane, DCM, methanol and water) of the stems of *Polyalthia suberosa* were employed as test material [14, 15]. Two gram-positive bacteria (*Staphylococcus aureus*, *Sarcina lutea*), four gram-negative bacteria (*Escherichia coli*, *Salmonella paratyphi*, *Vibrio mimicus* and *Shigella boydii*) and two fungi (*Aspergillus niger* and *Candida albicans*) were used as test microorganism. The test organisms were allowed to grow in nutrient agar medium on petri dishes at an incubator for 24 hours at 37 ˚C. Each sample had a concentration of 400µg/disc. Standard Ciprofloxacin (5µg/disc) discs and blank discs were used as positive and negative controls, respectively. The sample discs, the standard and the negative discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4 ˚C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37 ˚C for 24 hours. The antimicrobial potency of the test agents was measured by their activity to prevent the growth of the microorganism surrounding the discs which provided a clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zone of inhibition in millimeter (mm) with a transparent scale.

Free radical scavenging activity of *Polyalthia suberosa*

DPPH free radical scavenging activity method had been employed to determine the antioxidant activity of different extracts of *Polyalthia suberosa* stems [16-18]. Different concentrations of each extract were prepared using methanol by successive dilution method. Then 2 mL of each extract solution was mixed with 3 mL of methanolic DPPH solution (20µg/mL) and was kept for 30 minutes incubation period. The reaction progress was measured from bleaching of purple-colored methanol solution of DPPH radical by the plant extract in comparison to that of standard *tert*-butyl-1-hydroxytoluene (BHT) by UV spectrometer, where the absorbance was measured against a blank at 517 nm.

Free radical inhibition percentage (%) was calculated as follows:

$$(I\%) = (1-A_{sample}/A_{blank})\times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material). The antioxidant potential has been measured in terms of IC_{50} value, which is the concentration of the sample required to reduce 50% of DPPH free radical. IC_{50} was calculated from the graph plotted inhibition percentage against extract concentration.

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is a rapid general bioassay for the bioactive compound of the natural and synthetic origin [19, 20]. In this method, all the extracts (hexane, DCM, methanol and water) were tested for toxicity against brine shrimp nauplii (*Artemia salina*) in a 1-day *in vivo* bioassay method.

In the experiment, 4 mg of each extract was dissolved in 200 µL of pure DMSO in vials to get stock solutions. Solutions of varying concentrations as 400, 200,100, 50, 25. 12.5, 6.25, 3.125, 1.5625, 0.7812 and 0.3906 µg/mL were obtained by using serial dilution method adding 100 µl sample and 100 µL DMSO each time to the vial. The same procedure was followed to produce different solutions of vincristine sulphate, which had been used as a standard here. All the vials were observed for 24 hours. The cytotoxic activity was measured in terms of LC_{50} value (the concentration at which 50% mortality of brine shrimp nauplii occurred) that was obtained from the plot of log of concentration (log C) versus percent mortality.

Total antioxidant capacity

The phosphomolybdenum method was used for the determination of the total antioxidant capacity of different extracts of the plant [21-23]. An aliquot of prepared standard solutions of ascorbic acid was taken in an Eppendorf tube mixing with 6 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the capped tubes were incubated in a water bath at 95 ˚C for 90 minutes. After cooling the absorbance of each of the standard solutions was measured at 695 nm against a blank. The absorbance for prepared extract solutions (2 mg extract/ 10 mL solvent) was then recorded in the same procedure. The antioxidant capacities of different extracts were expressed as equivalents of ascorbic acid (AAE).

Results and Discussion

Phytochemical screening

Phytochemical screening carried out on plant sample showed the presence of several medicinally active secondary metabolites that have been enlisted in Table 1.

Table 1: Qualitative analysis of the phytochemicals of *Polyalthia suberosa*

Plant	Alkaloid	Terpenoid	Flavonoid	Steroid	Tannin	Saponin	Cardiac glycoside
Polyalthia suberosa	+	+	+	+	+	+	-

*Positive sign (+) indicates the presence and negative sign (-), the absence of phytochemicals

Antimicrobial screening

The results of antimicrobial screening using disc diffusion method showed the following result given in Table 2. The antimicrobial activity of all these extracts had been determined by comparing the zone of inhibition in mm produced by them to that of standard discs of Ciprofloxacin. The zones of inhibition produced by hexane, DCM and methanol ranged from 9-11mm, at a concentration of 400

µg/disc, where standard discs of Ciprofloxacin (5 µg/disc) produced 40-41mm. The water extract exhibited no inhibitory activity against tested bacteria and fungi. All these extracts except water extract moderately inhibited the growth of all gram-positive and gram-negative bacteria and fungi, but the most satisfactory result was found for methanolic extract (9-11mm).

Table 2: Antimicrobial activity of the extracts of the stems of *Polyalthia suberosa*

Test Organism	Zone of Inhibition (mm)				
	Hexane Extract 400 µg/disc	DCM Extract 400 µg/disc	Methanol Extract 400 µg/disc	Water Extract 400 µg/disc	Ciprofloxacin 5µg/disc
Gram-Positive Bacteria					
Staphylococcus aureus	10	10	10	-	40
Sarcina lutea	9	10	10	-	40
Gram-Negative Bacteria					
Escherichia coli	10	10	11	-	41
Salmonella paratyphi	9	10	10	-	41
Vibrio mimicus	10	10	10	-	41
Shigella boydii	10	10	10	-	41
Fungi					
Aspergillus niger	9	10	10	-	40
Candida albicans	9	10	9	-	40

Free radical scavenging activity

The antioxidant activity of the extracts (hexane, DCM, methanol and water) of the stems of *Polyalthia suberosa* was studied on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). IC_{50} value had been calculated from the plot of inhibition percentage vs. extract concentration and the results are given below (Table 3).

Results indicate that the hexane and DCM extracts of *Polyalthia suberosa* showed low antioxidant activity with high IC_{50} values i.e. 338.84 and 259.42 µg/mL respectively. The methanol and water extracts were found to exhibit high free radical scavenging activity with low IC_{50} values; 12.79 µg/mL and 19.95µg/mL, respectively. This indicates that these extracts may contain highly active free radical scavenging compounds which are polar in nature. So, further researches can be carried out to get more information about these activities.

Table 3: IC_{50} values of standard and different extracts of *Polyalthia suberosa* stems

Sample	IC_{50} (µg/mL)
Hexane Extract	338.84
DCM Extract	259.42
Methanol Extract	12.79
Water Extract	19.95
Tert-butyl-1-hydroxytoluene (BHT)	27.29

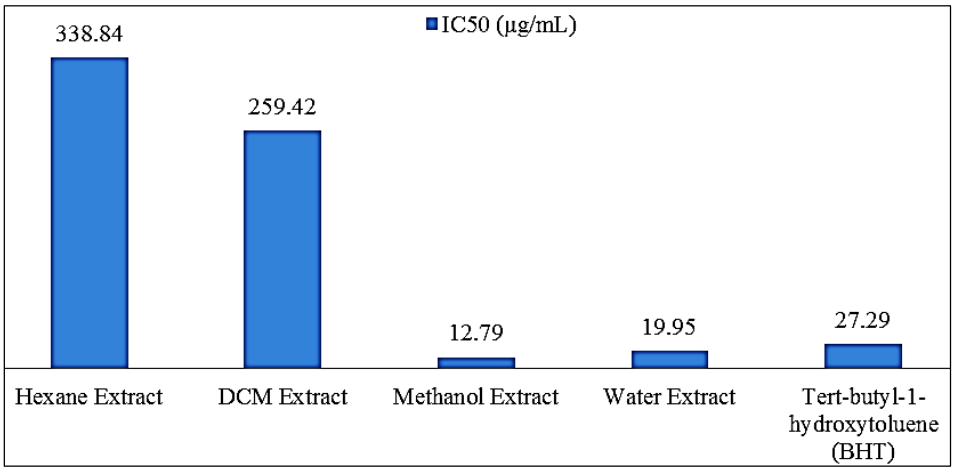

Fig 1: IC_{50} values of different extracts of *Polyalthia suberosa*

Brine shrimp lethality bioassay

The cytotoxic activity of the four extracts (hexane, DCM, methanol and water) was evaluated applying those over *Artemia salina* nauplii keeping for 24 hours. The activity was measured in terms of LC_{50} value using Vincristine sulphate as standard, which had been obtained from the plot of logC vs percent mortality for all test samples. Crude extracts of hexane, DCM, methanol and water showed potential cytotoxic activity with LC_{50} values 0.983, 0.251, 0.045 and 25.57 µg/mL, respectively (Table 4). From this result, DCM and methanol extracts were found to be the most effective with the lowest values of LC_{50} and we can conclude that these might contain compounds having antitumor or pesticidal properties and can be used as a good source of potent drugs.

Table 4: LC_{50} values of standard and different extracts of the stems of *Polyalthia suberosa*

Sample	LC_{50} (µg/mL)
Hexane Extract	0.983
DCM Extract	0.251
Methanol Extract	0.045
Water Extract	25.57
Vincristine Sulphate	0.719

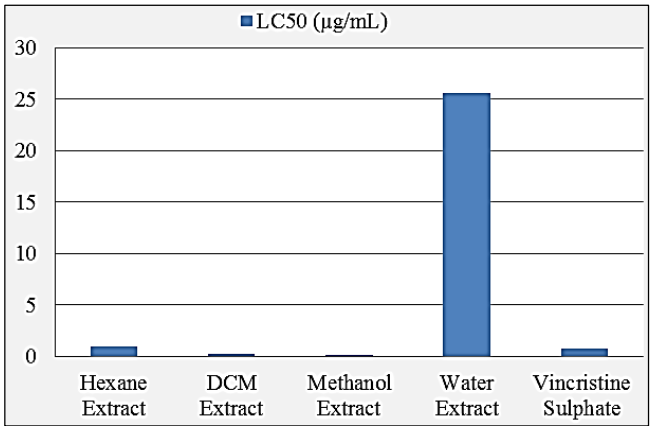

Fig 2: LC_{50} values of different extracts of *Polyalthia suberosa*

Total antioxidant capacity

In phosphomolybdenum method, the total antioxidant capacity of test extracts (hexane, DCM, methanol and water) was obtained by a plot of absorbance against concentration and the best-fit line was obtained from the curve data by means of regression analysis. Total antioxidant capacity of hexane, DCM, methanol and water extracts of *Polyalthia suberosa* was found to be 56.55, 74.82, 124.76 and 95.63 ppm ascorbic acid equivalent (AAE) respectively (Table 5). The highest value was found for methanolic extract, which means that it possesses higher antioxidant capacity, although other extracts also showed moderate antioxidant capacity.

Table 5: Total antioxidant capacity (equivalent ascorbic acid) of different extracts of the stems of *Polyalthia suberosa*

Sample	Total Antioxidant Capacity (in AAE) ppm
Hexane extract	56.55
DCM extract	74.82
Methanol extract	124.76
Water extract	95.63

From this result, it can be concluded that all these extracts of *Polyalthia suberosa* have high antioxidant capacity and hence, can be used as a potential source of useful drugs.

Conclusion

Phytochemical screening of aqueous extract and raw powder of the stems of *Polyalthia suberosa* showed the presence of potent secondary metabolites that have well known medicinal activities. Antimicrobial screening carried out on four different extracts of *Polyalthia suberosa* stems using disc diffusion method showed significant antimicrobial activity against tested bacteria and fungi for all the extracts except water extract. But the most effective result was reported for methanolic extract. The free radical scavenging activity of different extracts of the stems of *Polyalthia suberosa* using DPPH depicted methanol and water extracts to exhibit the most potent activity, while the other two extracts were found to provide low activity with high values of IC_{50}. The brine shrimp lethality bioassay using different extracts of plant stems showed effective mortality rates against brine shrimp nauplii with low LC_{50} values. The total antioxidant capacity of different extracts using the phosphomolybdenum method showed moderate to a good range of antioxidant capacity but the most potent result was found for methanolic extract.

All these results imply that the stems of *Polyalthia suberosa* contain highly potent antimicrobial, cytotoxic and antioxidative agents from which we can generate effective newer drugs to medicinal industry and carry out further research for more resourceful findings.

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