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Preliminary phytochemical screening, antibacterial activity and cytotoxic activity of leaves extract of *Carissa carandas* Linn.

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Abstract
Due to drug resistance to microbes, the necessity of investigation of medicinal plant for antibacterial activity is very crucial. Besides, medicinal plants may be a good starting point for discovering new chemotherapeutics. The aim of the present study comprehends investigation of preliminary phytochemical screening, antibacterial and cytotoxic activity of 60% ethanolic extracts of *Carissa carandas* Linn. leaves. Phytochemical screening was accomplished by employing standard methods. Antibacterial activity was investigated by the use of disc diffusion method and cytotoxic activity was investigated using brine shrimp lethality bioassay. The antibacterial activity of the extract was very poor. But the cytotoxic activity of the extract was good compared to the standard drug vincristine sulphate.

Keywords: *Carissa carandas* Linn, antibacterial activity, cytotoxic activity, zone of inhibition

Introduction
Use of plant extract to treat various disease have been increasing due to having secondary metabolites. Medicinal plants are potential source of drugs. Day by day popularity as well as acceptability of herbal medicines are increasing also because of having less side effects [1]. The characteristic of *Carissa carandas* Linn. (Apocynaceae) is white latex which is seen when the leaves or stems are injured [2]. Various chemical constituents such as carissol, carissic acid, ascorbic acid, lupenol, beta sitosterol, serine, glutamine, alanine, valine, phenylalanine and glycin were contained in this plant [3]. Leaves of this plant were shown to have steroids, glycosides, flavonoids, tannins, terpenoids and carbohydrates in the previous study [4]. The plants can be used as traditional medicine [5]. Leaves are used to treat fevers, diarrhoea, earache, syphilitic pain and snake poisoning [6]. In previous study it has also been showed that crude extract of leaves showed dose dependent antihyperglycemic activity [7]. For cytotoxic activity phenolics as well as flavonoids may be responsible constituents and for antibacterial activity tannins, triterpenoids, flavones may be responsible constituents [8]. Besides drug resistance against microbes is a major problem. For the effective control of microbial infections plant products are best option due to less side effects [9]. For discovering new drugs (chemotherapeutics) medicinal plant is a good starting point [10]. The objective of the present study was to investigate the antibacterial and cytotoxic activities of 60% ethanolic extract of leaves of *Carissa carandas* Linn.

Materials and methods
Collection of plant material
The leaves of *Carissa carandas* Linn. were collected from a village of Comilla, Bangladesh. Then the plant was authenticated from Bangladesh National Herbarium, Bangladesh, by a taxonomist. The accession number was 46724.

Extraction procedure
After shade drying of fresh mature leaves they were comminuted. Then the powdered material was dissolved for 24 hours into Petroleum ether (40^0-$60\ ^0$C) in order to remove chlorophyll. After completely removal of the solvent from the residue powdered material it was again dissolved into 60% ethanol for seven days with occasional stirring. The filtrate collected by filtration with a plug of cotton followed by filtration of filter paper. From the filtrate the solvent was evaporated using rotary evaporator. After air drying of the obtained dense mass the crude extract was obtained which was further used for the investigations.

Phytochemical Screening

A useful means of preliminary determination of nature of chemical constituents present in plant samples is phytochemical screening. Depending on availability of chemical reagents the test for carbohydrate (Benedict's test), test for reducing sugar (Fehling's test), test for saponin (Frothing test), test for flavonoid (Concentrated HCl acid), test for tannin ($FeCl_3$ test and lead acetate test) and test for phenol (5% aqueous $FeCl_3$ test) were performed for qualitative determination of chemical constituents by using the standard methods [11, 12].

Antibacterial activity

The antibacterial activity was meted using disc diffusion method following a standard method. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholera* and *Escherichia coli* were used as test bacteria. The bacteria were collected from the Department of Microbiology, Primeasia University, Bangladesh. Culture media was prepared using nutrient agar in petridishes and bacterial inoculum was poured and spreaded in zigzazg pattern over the solidified agar media. Sterile filter paper disks (6 mm diameter) were impregnated with 50 µl of 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.8125 mg/ml and 3.90625 mg/ml extract solution. Then the disc impregnated with extract and standard kanamycin (30 µg /disc) (oxoid, UK) were placed on the culture media. The culture media was incubated at 37^0 C for 24 hours keeping the petridishes invertedly. The zone of inhibition was measured in millimitre using a roller [1].

Cytotoxic activity

The Crude 60% ethanolic extract of *Carissa carandas* Linn. was meted for cytotoxic activity using brine shrimp lethality bioassay. The study was substantiated by following standard method of Meyer with minute modification. Brine shrimp eggs were collected from a pet merchandise and hatched in artificial sea water with continuous light source and oxygen supply for 48 hours to get nauplii by dissolving 38 gm iodine free salt in 1000 ml distilled water. Ten matured nauplii were taken in each test tubes containing 5 ml of artificial sea water. The crude extract sample solution of several concentration was added into the previously numbered test tubes using a micro pipette. Several concentrations of Vincristine sulphate was used as standard cytotoxic agent dissolved in DMSO. The study was fulfilled using three replicates for each concentration. The living shrimps were counted after 24 hours using a magnifying glass. After enumeration of % mortality it was plotted against log C in Microsoft Office Excel. From the regression line equation LC_{50} was enumerated. [13].

Result and Discussion

The results of qualitative phytochemical analysis of 60% ethanolic leaves extract of *Carissa carandas* Linn. are represented in Table 1.

Table 1: Results of Phytochemical analysis

Phytoconstituents	Observation
Carbohydrate	+
Reducing Sugar	-
Saponin	+
Flavonoid	+
Tannin	+
Phenol	+

Here plus (+) sign and and minus (-) sign indicates the presence and absence of phytoconstituents respectively.

In the previous study it had been found that various extracts of leaves of the plant contained carbohydrates, steroids, glycosides, cardiac glycosides, flavonoids, tannins, terpenoids, phenolic compounds, saponins, proteins, alkaloids, oil and fats, but reducing sugar was absent [4, 14]. In the present study 60% ethanolic extract was meted for the presence of carbohydrate, reducing sugar, saponin, flavonoid, tannin and phenol based on the availability of reagents. All of the investigated phytoconstituents were present in the extract except reducing sugar. The result supports the previous study.

The zone of inhibition of extract as well as standard Kanamycin on four bacteria obtained after overnight incubation are represented in table 2 and figure 1.

Table 2: Zone of inhibition in mm of *Carissa carandas* Linn. as well as Kanamycin

Test organisms	Zone of inhibition in mm							
	Carissa carandas Linn. (mg/ml)							Kanamycin (30 µg/disc)
	250	125	62.5	31.25	15.625	7.8125	3.90625	
V. cholerae	-	-	-	-	-	-	-	18
P. aeruginosa	-		-	-	-	-	-	23
S. aureus	10	9	-	-	-	-	-	19
E. coli	-	-	-	-	-	-	-	23

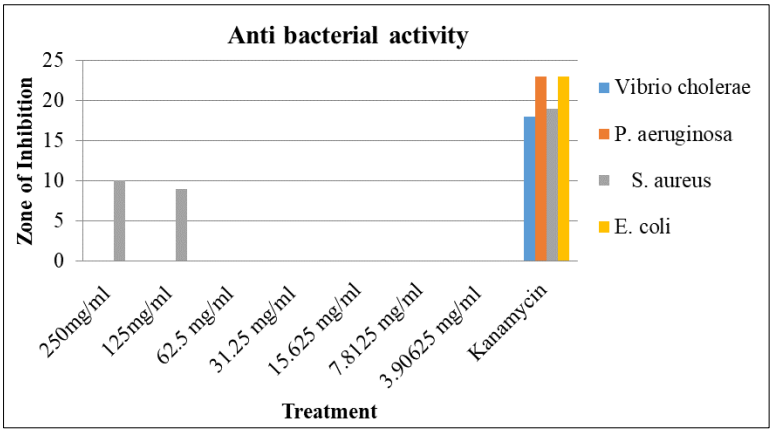

Fig 1: Antibacterial activity of *Carissa carandas* Linn. as well as standard Kanamycin

From the table and graphical representation it can be seen that the extract showed zone of inhibition of 10 mm and 9 mm only against *S. aureus* at the dose of 250 mg/ml and 125 mg/ml respectively. The standard drug Kanamycin showed good zone of inhibition against all of the test bacteria. So it can be said that the antibacterial activity of leaves extract in this present study was very poor.

Effect of 60% ethanolic extract as well as Vincristine sulphate on brine shrimp nauplii has been tabulated in table 3 and table 4 as well as graphically represented in figure 2 and figure 3 after 24 hrs.

Table 3: Effect of *Carissa carandas* Linn. as well as Vincristine sulphate on brine shrimp nauplii

***Carissa carandas* Linn.**			**Vincristine sulphate**		
Concentration (µg/ml)	**Log C**	**%Mortality**	**Concentration (µg/ml)**	**Log C**	**%Mortality**
400	2.602	100	40	1.602	100
200	2.301	90	20	1.301	100
100	2	66.67	10	1.000	100
50	1.699	76.67	5	0.698	70
25	1.398	43.33	2.5	0.397	60
12.5	1.097	53.33	1.25	0.096	60
6.25	0.796	40	0.625	-0.204	70
3.125	0.495	26.67	0.3125	-0.505	70
1.563	0.195	30	0.15625	-0.806	60
0.781	-0.107	20	0.078125	-1.107	40

Table 4: LC_{50} value of extract as well as Vincristine sulphate

Sample	**LC_{50} (µg/ml)**	**Regression line equation**	**R^2**
Carissa carandas Linn.	12.6	y=28.99x + 18.49	R^2 = 0.914
Vincristine sulphate	0.12	y=19.53x+68.17	R^2 = 0.748

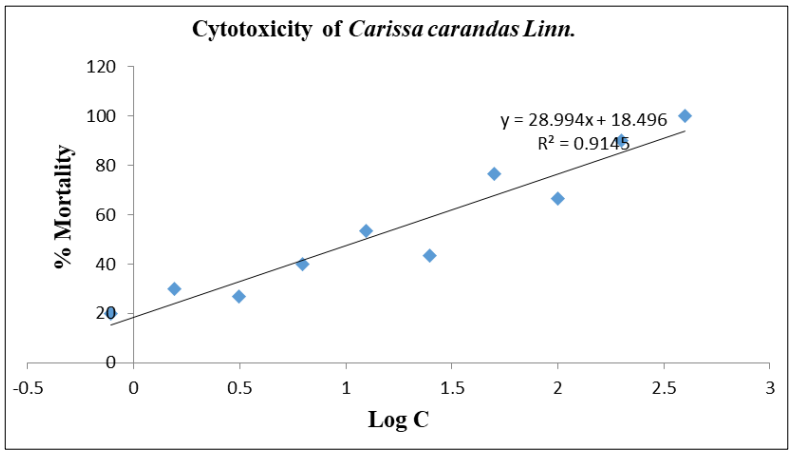

Fig 2: Plot of Log C versus % mortality of shrimp after 24 hours

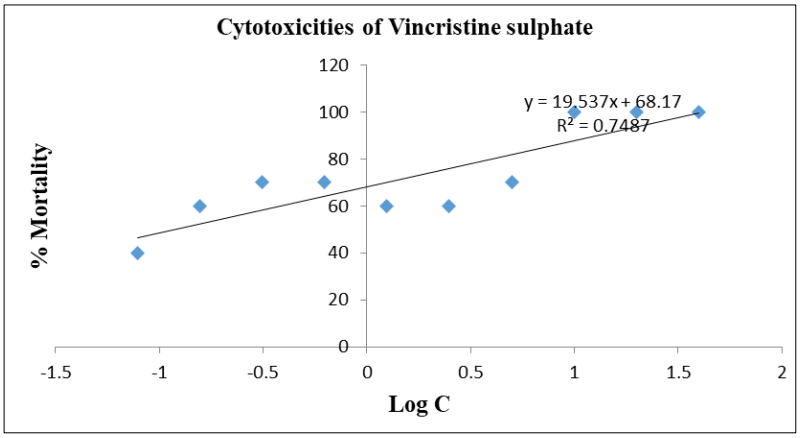

Fig 3: Plot of Log C versus % mortality of shrimp after 24 hours

From the table and graphical representation it can be seen that the % mortality of extract as well as standard vincristine sulphate was increased with the increasing concentration. LC_{50} value was determined from the equation of regression line. LC_{50} value of extract was 12.6 µg/ml. Whereas the LC_{50} value of standard vincristine sulphate was 0.12 µg/ml. From the previous study it can be portrayed that flavonoids and phenolics possessed poisonous effect on cell mytosis. So these compounds are responsible for cytotoxic activity [15]. Flavonoid and phenol present in the extract to be explored in the present study might be responsible for the cytotoxicity.

Conclusion

The 60% ethanolic extract of *Carissa carandas* Linn. Possess very poor antibacterial activity compared to the standard drug. On the contrary, the cytotoxic activity of this extract was good compared to the standard drug. Further study can be performed in order to separate the compounds responsible for cytotoxic activity. These had been concluded by the present study.

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