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ASSESSMENT OF ANTIOXIDANT, ANTIBACTERIAL AND PRELIMINARY CYTOTOXIC ACTIVITY OF CHLOROFORM AND METHANOL EXTRACTS OF *CAESALPINIA CRISTA* L. LEAF

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Abstract

This study was carried out to elucidate the potential antioxidant, antibacterial and preliminary cytotoxic activity of chloroformic and methanolic leaf extracts of *Caesalpinia crista* L. in different established *in vitro* experimental methods. The methanolic extract showed moderate 50% inhibitory concentration (IC_{50}) value (103.7 µg/ml) in DPPH assay. Reducing power increased in a concentration-dependent manner while significant total antioxidant capacity (70.4 mM Fe (II)/g) was observed in methanolic extract using FRAP assay. Methanolic leaf extracts of *C. crista* showed to possess considerable amount of phenol (52.5 mg GAE/g), flavonoid (100.4 mg QE/g) and tannin (76.6 mg GAE/g). The methanolic extracts also exhibited the zone of inhibition at 250 and 500 µg/disc, respectively, against several pathogenic bacterial strains while the MIC values rangeed from 62.5 to 500 µg/ml. The significant 50% lethal concentration (LC_{50}) of chloroformic and methanolic extract, respectively was found against brine shrimp nauplii (5.794 and 2.972 µg/ml). The results suggested that the methanolic leaf extract of *C. crista* possessed potential antioxidant, antibacterial and preliminary cytotoxic activity.

Introduction

Natural sources are limitless inspiration for novel drug development because there are many promising drug candidates available (Newman and Cragg 2012). Plants and their extracts are an important element of indigenous medical systems and they serve the primary health care needs of more than 80% of the people in the world (Hassan *et al.* 2009). Again, plant derived biological active compounds have been the single most productive source of leads for the development of modern medicine.

Caesalpinia crista L. of Fabaceae is a prickly shrub well-known medicinal plant in tropical and subtropical regions of Southeast Asia (Williamson 2002). The plant is locally known as Kutum Kanta in Bangladesh (Hasan 2000) while it is well-known as a Natakaranja or Katikaranja in India (Sarkar *et al.* 2012). Traditionally, various plant parts of *C. crista* are used as antipyretic, periodic, tonic and vesicant for the treatment of gynecological disorders, skin diseases, constipation, piles, ulcers, rheumatism and backache (Williamson 2002). The different extracts of *C. crista* have been reported to have anthelmintic activity (Jabbar *et al.* 2007), antimalarial activity (Kalauni *et al.* 2006), antioxidant activity (Mandal *et al.* 2011), anti-amyloidogenic (Ramesh *et al.* 2010), nootropic activity (Kshirsagar 2011) and cytotoxic activity (Bodakhe *et al.* 2011, Sharkar and Mandal 2011). Literature on isolated compounds from *C. crista* suggests most of the compounds belong to diterpenes group. However, most of the previous studies included a single

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antioxidant platform using a solvent while the present study incorporated six different antioxidant assays in two different solvent systems. In addition, it also reports the determination of MIC values of these extracts for the first time. Therefore, the present study aims at evaluating the antioxidant, antibacterial and cytotoxic activity from chloroformic and methanolic leaves extract of *C. crista*.

Materials and Methods

The plant material of *Caesalpinia crista* was collected from Dhangmaree, Chadpai Range of the Sundarbans East Division situated in Bagerhat district, Bangladesh on 16th December, 2011 and sent to Bangladesh National Herbarium for identification. After washing with distilled water, the collected leaf samples were shed dried. The dried material was ground into powder and stored in airtight containers. Petroleum ether, chloroform and methanol were used as solvents. About 125 gm of powdered *C. crista* leaf was soaked into 450 ml petroleum ether for a 5 days with occasional stirring and shaking. It was then filtered and after this first filtration, the remaining residues (approx. 120 gm powder) were soaked into 400 ml chloroform, kept for a period of 6 days and then filtered and the final remaining residues (117 gm powders) were soaked into 390 ml methanol respectively, kept for 6 days and then filtered. These extracts were passed through filter paper, and the filtrates were evaporated, yielding the petroleum ether, chloroformic and methanolic extracts, respectively. However, the amount of petroleum ether extracts was insufficient, for that reason methanolic and chloroformic extracts were used.

In determining DPPH free radical scavenging activity, different concentrations of the extracts (57 - 400 µmg/l) was prepared and then 2 ml of 0.004% DPPH solution was added in each test tube and kept at dark for 30 min to complete the reaction and then absorbance was recorded at 517 nm (Gupta *et al.* 2003). Control was prepared in the same way as the sample except addition of sample or standard. Per cent scavenging activity was calculated using the formula: Scavenging activity = $(A_0 – A_1)/A_0 \times 100\%$, where A_0 is the absorbance of control, and A_1 is the absorbance of sample or standard. Mean values were obtained from triplicate experiments.

Reducing power was determined according to the method stated by Oyaizu (1986) while **t**otal antioxidant activity in the form of the FRAP assay was carried out according to the method reported by Benzie and Strain (1996). Total phenolic content and total flavonoid content of the extracts were determined by Folin-Ciocalteau assay and aluminium chloride colorometric assay (Peteros and Mylene 2010), respectively with slight modifications. Gallic acid and quercetin were used as standard, respectively. Total tannins content in plant extract was determined by Folin-Denis method as described by Polshettiwar and Ganjiwale (2007) with slight modifications.

Antibacterial activity of *C. crista* extracts was tested by disc diffusion method (Bauer *et al.* 1966). Five Gram positive and five Gram negative bacterial strains (Table 2) were maintained on the nutrient agar medium. The sterile filter paper discs were prepared by adding desired concentration (250 and 500 µg/disc) of extracts on the disc. Standard tetracycline disc (30 µg/disc), discs containing extracts and control discs were then impregnated, incubated overnight at 37˚C, checked for the zone of inhibitions and measured in millimeters (mm). Measurements were taken in triplicate. The extracts that showed antimicrobial activity in disc diffusion were later tested to determine the MIC value for each bacterial sample by using broth macro-dilution method (Nascimento 2000) according to the Clinical and Laboratory Standards Institute (CLSI) protocol (Wayne 2010). Briefly, bacterial samples were grown in nutrient broth for 6 hrs. Approximately 100 µl of these cultures containing 10^6cells/ml was inoculated in separate tubes with nutrient broth supplemented with different concentration of the extracts ranging from 62.5 to 500 µg/ml. Afterwards 24 hrs incubation at 37°C, the MIC of each sample was determined by measuring the

optical density in the spectrophotometer (620 nm), comparing the sample readout with the non inoculated nutrient broth.

Preliminary cytotoxic activity of the extracts was determined by the method described by Apu *et al.* (2010). Vincristine sulphate was used as positive control. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC_{50} were calculated using Graphpad Prism Version 6.01 (GraphPad Software, Inc., USA).

Results and Discussion

DPPH free radical scavenging and reducing power of chloroformic and methanolic leaf extracts of *C. crista* are shown in Fig. 1A, B), respectively. The chloroformic and methanolic extract of *C. crista* leaf showed IC_{50} value of 201.92 and 103.7 µg/ml, respectively and that for the standard quercetin was 7.62 µg/ml. Activity was increased linearly in a concentration dependent manner at lower concentration and saturation reached at higher concentration for both the extracts and standard. Chloroformic extract of *C. crista* leaf has a smaller antioxidant potential, may be due to the presence of non-polar or less polar compounds. Methanolic leaf extract of *C. crista* showed the lowest IC_{50}, which means that it possessed the strongest DPPH free

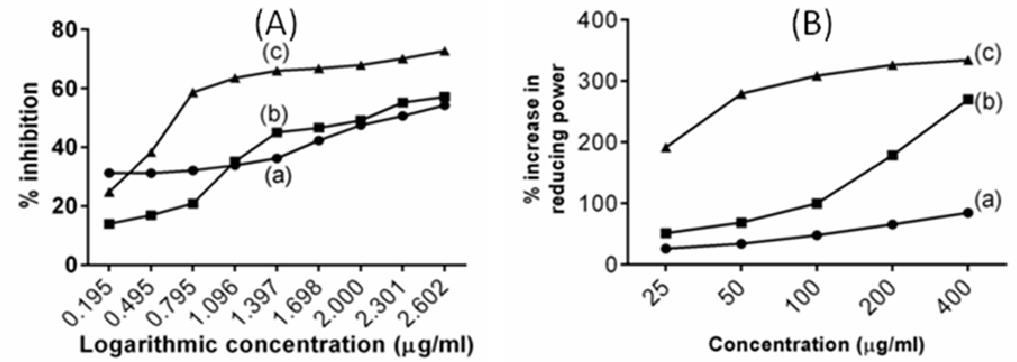

Fig.1.A: DPPH-scavenging activity of chloroformic (a) and methanolic (b) extract of *C. crista* leaf in comparison with standard quercetin (c), B: Reducing power of chloroform (a) and methanol (b) extract of *C. crista* leaf in comparison with standard Quercetin (c).

radical scavenging activity. It was previously reported that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds, and aromatic amines reduce and decolorize DPPH by their hydrogen donating ability (Blois 1958, Hossain *et al.* 2015). Therefore, methanolic leaf extracts seem to possess hydrogen donating capability as they tend to extract mostly polar compounds from the plant material. It was also in good agreement with the findings of Mandal *et al.* (2011), who reported that methanolic extract of *C. crista* leaves acts as an antioxidant and ROS (reactive oxygen species) scavenger. The activity of antioxidants is associated with reducing power (Meir *et al.* 1995, Hossain *et al.* 2015). Table 1 shows the reducing power of methanolic and chloroformic leaf extracts as determined by potassium ferricyanide reduction method. At a concentration of 400 µg/ml, chloroformic and methanolic leaf extracts showed high reducing power as represented by optical density of 0.647 ± 0.21 and 1.271 ± 0.084, respectively. The reducing power of the extracts seemed to increase in a dose-dependent manner (Fig. 1B). Potential activity was found in methanolic leaf extracts, which increased up to 265% of the initial value. These could be attributed due to the influence of solvent system (Moure *et al.* 2001) and

presence of active reductants in the plant extracts (Sghaier *et al.* 2012). Total antioxidant power expressed as quercetin equivalent (QE), total phenolic content as gallic acid equivalent (GAE), total flavonoids as QE, total tannins as GAE, are shown in Fig. 2A-D, respectively. Chloroformic leaf extract of *C. crista* showed the antioxidant capacity of 35.4 µM Fe (II)/g sample while methanolic extract showed the potential antioxidant capacity of 70.4 µM Fe (II)/l (Table 1). The total phenolic content of chloroformic and methanolic leaf extract was found to be 7.7 and 52.5 mg GAE/g of dried plant materials, respectively (Table 1). The values for total flavonoids of chloroformic and methanolic leaf extract were found to be 41 and 100.4 mg of QE/g of dried leaf extract, respectively (Table 1). The total tannin content of chloroformic and methanolic leaf extract was found to be 9 and 76.6 mg GAE/g of dried leaf extract, respectively (Table 1).

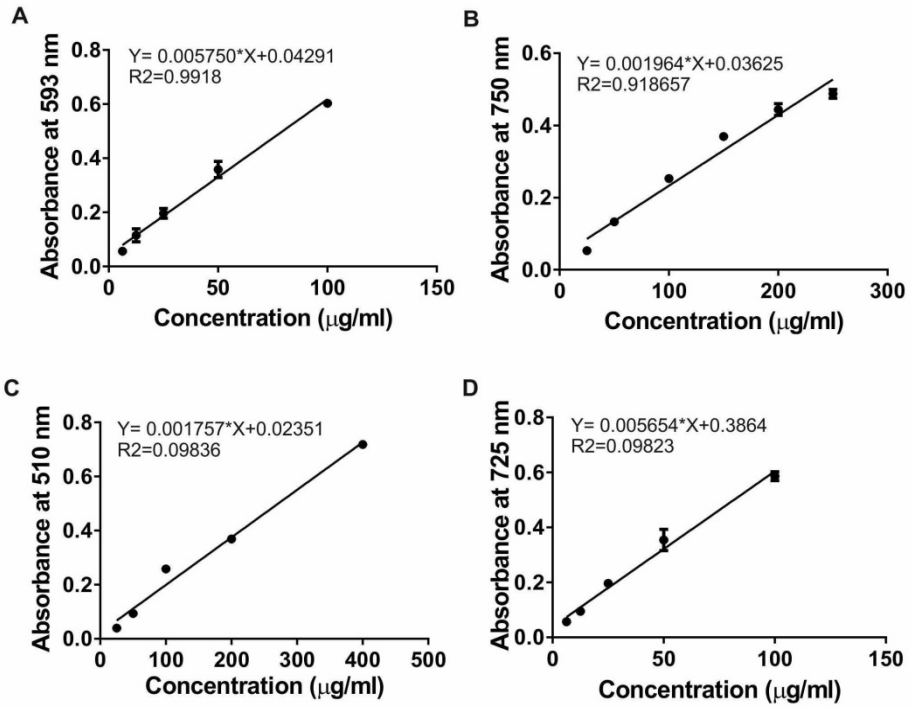

Fig. 2. Standard calibration curve of: A: Quercetin for determining total antioxidant power, B: Gallic acid to estimate total phenolic content, C: Quercetin to measure total flavonoid content and D: Gallic acid to determine total tannin content of *C. crista* leaf, respectively.

Methanolic leaf extract of *C. crista* produced zone of inhibition against seven bacterial strains (Table 2). Zone of inhibition ranged between 7.6 and 11.5 and 11 to 14.8 mm, at the doses of 250 and 500 µg/disc, respectively. Methanol extract at 500 µg/disc showed the highest antibacterial activity against *S. aereus* and *E. coli* (14.8 mm and 14.5 mm diameter zone of inhibition, respectively). However, the chloroformic leaf extract failed to demonstrate any significant zone of inhibition against all the tested bacterial strains. The MIC values (µg/ml) of the leaf extracts against the experimental organisms obtained are shown in Table 2. The results revealed variability in the inhibitory concentrations of methanolic and chloroformic leaf extracts of *C. crista* against tested bacteria. The lowest MIC value was found to be that of methanolic leaf extract (159.5 µg/ml) against *S. aereus* ATCC 25923 while the highest MIC value (443 µg/ml) was found against

Micrococcus. Methanolic leaf extract exhibited significant antibacterial activity against all Gram positive bacterial strains and two Gram negative bacterial strains. Polarity of the compounds affects the diffusion of natural products in culture medium. Less polar compounds diffuse more slowly and poorly than polar compounds, because agar media is prepared in water (Moreno *et al.* 2006).

Table 1. Showing the results related to DPPH-free radical scavenging power assay, reducing power, total antioxidant capacity, total phenolic, flavonoid and tannin content of chloroformic and methanolic leaf extracts of *C. crista.*

Name of the tests	Solvent system	Values in corresponding units	Regression equation	R^2 value
DPPH (IC_{50} value in µg/ml)	Ch	201.9	y = 11.76x + 22.89	0.971
	Mt	103.7	y = 19.66x + 10.35	0.943
	Quercetin	7.62	y = 17.09x + 34.91	0.749
Total antioxidant capacity (µM Fe (II)/g)	Ch	35.4 ± 0.023	Quercetin calibration curve : y = 0.005x + 0.042	0.991
	Mt	70.4 ± 0.031		
Total phenolic content (mg GAE/g of dry plant material)	Ch	7.7 ± 0.013	Gallic acid calibration curve : y = 0.002x + 0.036	0.968
	Mt	52.5 ± 0.012		
Total flavonoid content (mg QE/g of dry plant material)	Ch	41 ± 0.009	Quercetin calibration curve : y = 0.001x + 0.023	0.984
	Mt	100.4 ± 0.008		
Total tannin content (mg GAE/g of dry plant material)	Ch	9 ± 0.006	Gallic acid calibration curve : 0.005x + 0.037	0.988
	Mt	76.6 ± 0.011		
Reducing power (highest value at maximum conc.)	Ch	0.647 ± 0.021	Standard (quercetin) : 1.673 ± 0.012	
	Mt	1.271 ± 0.084		

Table 2. Results of disc assay of methanolic leaf extracts of *C. crista* and MIC values of the extracts against ten bacterial strains.

Tested organism	Methanolic leaf extract		Positive control (tetracycline)	MIC (µg/ml)
	250 µg/disk	500 µg/disk	30 µg/disk	
Gram positive bacteria				
B. cereus (ATCC 14579)	10 ± 1	13.5 ± 0.5	42	247
B. megaterium (ATCC 18)	9.3 ± 0.57	12.3 ± 0.57	45	319
B. suntilis (ATCC 6059)	8.6 ± 1.5	11 ± 0	35	300.7
Micrococcus	7.6 ± 0.57	11.1 ± 1.25	44	443
S. aereus (ATCC 25923)	11 ± 0	14.83 ± 0.76	30	159.5
Gram negative bacteria				
E. coli (ATCC 8739)	11.5 ± 0.5	14.5 ± 0.5	30	192.2
Klebsiella (ATCC 700603)	-	-	-	-
P. aeruginosa (ATCC 27833)	10 ± 0.5	13 ± 2	32	301.3
S. dysenteriae (ATCC 26131)	-	-	42	-
S. typhi (ATCC 13311)	-	-	30	-

Data are presented as mean measurement of zone of inhibition (mm), if not mean ±SD; ATCC: American type culture collection.

In brine shrimp lethality bioassay, LC_{50} values of chloroformic and methanolic leaf extracts of *C. crista* was found to be 5.794 and 2.972 µg/ml, respectively, compared to positive control vincristine sulphate (VS) with a LC_{50} value of 0.128 µg/ml (Table 3). Per cent mortality of methanolic leaf extract of *C. crista* was proximal to the standard indicating the strong cytotoxic activity of this extract (Peteros and Mylene 2010). The crude extracts resulting in LC_{50} values less than 250 µg/ml are considered significantly active (Kabir *et al.* 2012).

Table 3. Result of brine shrimp lethality bioassay of chloroformic and methanolic leaf extracts of *C. crista.*

Extract type	LC_{50} value (µg/ml)	Regression equation	R^2
Chloroformic leaf	5.794	y = 31.66x + 25.83	0.964
Methanolic leaf	2.972	y = 26.57x + 37.42	0.965
Control (vincristine sulfate)	0.128	y = 18.38x + 64.64	0.85

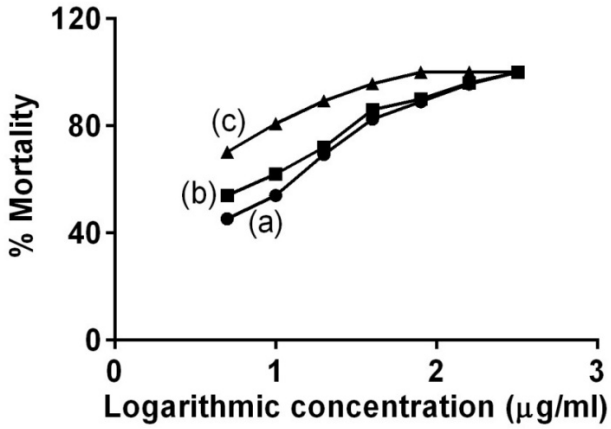

Fig. 3. Brine shrimp lethality bioassay of chloroform (a) and methanol (b) extract of *C. crista* leaf in comparison with standard vincristine sulfate, VS (c).

The methanolic leaf extract of *C. crista* showed notable antioxidant, and antibacterial potentials for its use in traditional medicine. Results demand further investigations to isolate bioactive compounds responsible for these bioactivities.

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