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Research Article

PRELIMINARY BIOLOGICAL INVESTIGATION OF TWO MEDICINAL PLANTS OF BANGLADESH

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ABSTRACT

The crude methanol extracts of whole plant of *Caladium bicolor* (Aiton) Vent. and leaf of *Chenopodium album* L. as well as their pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions were evaluated for antioxidant, cytotoxic and thrombolytic activities. The antioxidant potential was evaluated by DPPH and Folin-Ciocalteau reagent using butylated hydroxytolune (BHT) and ascorbic acid as standards. In DPPH free radical scavenging assay, the carbon tetrachloride soluble fraction of *C. bicolor* (IC_{50}= 124.21±0.43 µg/ml) demonstrated the highest free radical scavenging activity which could be correlated to its phenolic content 12.35±0.21 mg of GAE / gm of extractives. Among the test samples of *C. album, t*he highest free radical scavenging activity was demonstrated by the aqueous soluble fraction (IC_{50}= 31.49±1.21 µg/ml) with a phenolic content of 83.42±0.24 mg of GAE / gm of extractives. In brine shrimp lethality bioassay, the chloroform soluble fraction of *C. bicolor* and the pet-ether soluble fraction of *C. album* exhibited the highest cytotoxic activity with LC_{50} values 0.72±1.01 µg/ml and 0.54±0.41µg/ml, respectively. The chloroform soluble fraction of *C. bicolor* showed 37.93±0.91 % clot lysis as compared to 66.77% clot lysis by standard streptokinase while the crude methanol extract of *C. album* showed 24.25±1.03 % clot lysis.

Keywords: *Caladium bicolor* (Aiton) Vent., *Chenopodium album* L., DPPH, thrombolytic activity.

INTRODUCTION

Caladium bicolor (Aiton) Vent. (Synonyms: *C. picturatum, C. marmoratum)* commonly known as fancy leaf caladium, elephant's ear and hear of jesus, is flowering herb in the plant family Araceae. It is found from coastal Brazil to the Andes Mountains and north to the Guianas and Panama[1]. *Caladium bicolor* is found in Trinidad, an island very close to the South American mainland, and from the great variation found in the plants[2], the species appears to be native there. An infusion of fresh leaf is used for the treatment of angina. The powdered dried leaf is used to treat infected sores[3].

Chenopodium album L. (Synonyms: *Anserina candidans* Lam. Montandon., *Atriplex alba* L. Crantz, Bengali name: betho shaak) commonly known as lamb's quarters, melde, goosefoot and fat-hen, is a fast-growing weedy annual plant belonging to the Amaranthaceae family. Though cultivated in some regions, the plant is elsewhere considered a weed. The plant's native range includes most of Europe and is widely introduced in Africa, Australasia and North America. The plant improves appetite, acts as anthelmintic, laxative, diuretic and tonic. It is also used in abdominal pain, piles and eye disease[4]. The finely powdered leaves are used as a dusting powder about the external genitalia in children[5].

As part of our ongoing investigations on medicinal plants of Bangladesh[6, 7], the crude methanol extracts of whole plant of *C. bicolor* and leaves of *C. album* growing in Bangladesh as well as their organic and aqueous soluble fractions were studied for the antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic and thrombolytic activities for the first time and we,

here in, report the results of our preliminary investigations.

MATERIALS AND METHODS

Plant materials

The whole plant of *C. bicolor* and leaves of *C. album* were collected from Mirpur Botanical garden, Dhaka in January 2012. Voucher specimens for *C. bicolor* and *C. album* have been maintained in Bangladesh National Herbarium, Dhaka Bangladesh for future references.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (650 gm each) of both the plants were separately soaked in 1.5 liters of methanol at room temperature for 7 days. The extracts were filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature (40-45ºC) and pressure. An aliquot (5 gm) of each of the concentrated methanol extracts was fractionated by the modified Kupchan partition protocol[8] and the resultant partitionates were evaporated to dryness with rotary evaporator to yield pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble materials (Table 1). The residues were then stored in a refrigerator until further use.

Total phenolic content

The total phenolic content of the extractives was determined with Folin Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006)[9].

DPPH free radical scavenging assay

Following the method developed by Brand-Williams *et al*. (1995)[10], the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

Brine shrimp lethality bioassay

This technique was applied for the determination of general toxic properties of the DMSO solutions of plant extractives against *Artemia salina* in a single day *in vivo* assay[11]. Vincristine sulphate was used as positive control.

Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by Prasad *et al.* (2006)[12] by using streptokinase as positive control.

Statistical analysis

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

RESULTS AND DISCUSSION

The aim of the study was to evaluate the crude methanol extracts of *C. bicolor* and *C. album* as well as their pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions for antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic and thrombolytic activities.

In DPPH free radical scavenging assay, all the test samples of *C. bicolor* demonstrated mild free radical scavenging potential with IC_{50} values ranging from 124.21 µg/ml to 211.48 µg/ml. The highest free radical scavenging activity was demonstrated by the carbon tetrachloride soluble fraction (IC_{50}= 124.21±0.43 µg/ml) which could be correlated to its phenolic content 12.35±0.21 mg of GAE / gm of extractives. Highly significant free radical scavenging potentials were demonstrated by all the test samples of *C. album* with IC_{50} values ranging from 31.49 µg/ml to 125.30 µg/ml. The highest free radical scavenging activity was demonstrated by the aqueous soluble fraction (IC_{50}= 31.49±1.21 µg/ml) which could be correlated to its phenolic content 83.42±0.24 mg of GAE / gm of extractives (Table 2).

All the test samples of *C. bicolor* and *C. album* demonstrated significant cytotoxic potential against *A. salina.* The chloroform soluble fraction of *C. bicolor* and the pet-ether soluble fraction of *C. album* exhibited the highest cytotoxic activity with LC_{50} values 0.72±1.01 µg/ml and 0.54±0.41µg/ml, respectively as compared to 0.451 µg/ml for Vincristine sulphate (Table 2).

The extractives of *C. bicolor* and *C. album* demonstrated mild to moderate thrombolytic activity. The chloroform soluble fraction of *C. bicolor* showed 37.93±0.91 % clot lysis as compared to 66.77% clot lysis by standard streptokinase. The crude methanol extract and the chloroform soluble fraction of *C. album* showed 24.25±1.03 % and 23.80±0.94 % clot lysis, respectively (Table 3).

Table 1: Kupchan partitioning of *C. bicolor* and *C. album*

Crude extract/ Fractions	*C. bicolor* (gm)	*C. album* (gm)
Me	5.0	5.0
PESF	1.0	1.0
CTCSF	0.8	1.8
CSF	1.2	1.0
AQSF	1.5	0.5

ME= Methanolic crude extract; PESF= Pet-ether soluble fraction;
CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction;
AQSF= Aqueous soluble fraction.

Table 2: The total phenolic content, free radical scavenging and cytotoxic activities of *C. bicolor* and *C. album*

Plants	Samples/ Standards	Total phenolic content (mg of GAE/gm of extract)	DPPH Free radical scavenging activity (IC_{50} µg/ml)	Cytotoxic activity (LC_{50} µg/ml)
C. bicolor	ME	10.65±0.52	155.61±0.33	4.69±0.22
	PESF	1.27±0.29	211.48±0.60	103.44±0.68
	CTCSF	12.35±0.21	124.21±0.43	1.49±0.03
	CSF	7.75±0.61	150.73±0.56	0.72±1.01
	AQSF	-	-	-
C. album	ME	21.66±0.81	125.30±0.22	36.87±0.77
	PESF	61.32±1.24	32.20±0.34	0.54±0.41
	CTCSF	53.92±0.47	34.25±0.59	38.92±0.18
	CSF	48.73±0.39	49.85±0.11	25.46±0.69
	AQSF	83.42±0.24	31.49±1.21	24.48±0.51
Standards	VS	-	-	0.451
	BHT	-	27.5 ± 0.54	-
	Ascorbic acid	-	5.8 ± 0.21	-

BHT= Butylated hydroxytolune; VS= Vincristine sulfate; ME= Methanolic crude extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table 3: Thrombolytic Activity of *C. bicolor* and *C. album*

Samples/ Standard	% of lysis of RBCs	
	C. bicolor	*C. album*
ME	21.18±0.82	24.25±1.03
PESF	15.58±1.42	15.39±0.23
CTCSF	8.22±0.61	10.72±0.48
CSF	37.93±0.91	23.80±0.94
AQSF	-	11.59±0.55
Water	3.79 ± 0.55	
SK	66.77 ± 1.08	

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction;
CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; SK =Streptokinase

CONCLUSION

It is clearly evident from the above findings that the test samples of *C. bicolor* have significant cytotoxic activity. The plant also exhibited mild to moderate antioxidant and thrombolytic activities while the test samples of *C. album* have strong antioxidant and cytotoxic potentials. The plant also exhibited mild to moderate thrombolytic activity. Therefore, both the plants are good candidates for further systematic, chemical and biological studies to isolate the active principles.

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