

Antimicrobial, Antioxidant and Cytotoxic Activities of *Gomphrena globosa* (L.)

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The plant *Gomphrena globosa* grows well in different parts of Bangladesh and used traditionally for many medicinal purposes. To verify the traditional uses the crude methanol extract and its hexane, carbon tetrachloride, chloroform and aqueous soluble fractions were subjected to antimicrobial, antioxidant and brine shrimp lethality bioassays. Among all extractives the hexane soluble fraction exhibited the highest antioxidant activity having IC_{50} value 13.17 ± 0.308 $\mu\text{g/ml}$ and the crude methanol extract showed significant antioxidant activity with IC_{50} value 20.35 ± 0.360 $\mu\text{g/ml}$. Additionally, the chloroform soluble materials revealed significant cytotoxicity with LC_{50} of 0.331 ± 0.029 $\mu\text{g/ml}$. On the other hand the carbon tetrachloride and chloroform soluble partitionates demonstrated mild to moderate antimicrobial activity (zone of inhibition, 8 ± 0.208 to 14 ± 0.069 mm).

Gomphrena globosa (L.) (Bengali name Botam phul, Family- Amaranthaceae) commonly known as glove amaranth is an annual branched herb which is cultivated as ornamental flowering herb in garden (Muller and Borsch, 2005). It is native to North-America, South-America, Myanmar, and India and grows well over Bangladesh. Amaranthaceae is a large family comprising around 10 sub families, 176 genera and 2400 species available all over the world (Judd *et al.*, 2007; Kadereit *et al.*, 2006). *G. globosa* is a folk remedy for oliguria, heat and empacho, hypertension (Yusuf *et al.*, 2009), cough and diabetes (Arcanjo *et al.*, 2011) and expectorant for animals (Asolkar *et al.*, 1992). Since this plant has important medicinal properties, the present study has been undertaken and we, herein, report the antimicrobial, antioxidant and cytotoxic properties of *G. globosa* for the first time.

Whole plants of *Gomphrena globosa* (L.) were collected from Dhaka in November 2011. A voucher specimen (Accession no. 3557) for this collection has been

deposited in University of Dhaka herbarium for future reference.

The powdered whole plant (800 g) of *G. globosa* was extracted with 3.5 L of methanol for 15 days and filtered through a cotton plug. The extract was then concentrated with a rotary evaporator. An aliquot (5.0 g) of the concentrated aqueous methanol extract was fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) and subsequent evaporation of solvents afforded hexane (1.80 g), carbon tetrachloride (1.55 g), chloroform (0.5 g) and aqueous soluble (0.75 g) materials. Partitionates obtained by this way were subjected to different biological screenings.

The antimicrobial activity of the extractives was determined by the disc diffusion method (Austin *et al.*, 1999, Bauer *et al.*, 1966) against 5 gram positive bacteria, 8 gram negative bacteria and 3 fungal strains (Table 1). The organisms were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The experiments were carried out in triplicate using ciprofloxacin and kanamycin as standard and the results have been shown as mean \pm SEM (standard error of mean).

For cytotoxicity screening, the hexane, carbon tetrachloride and chloroform soluble materials of crude methanol extract were separately dissolved in DMSO (Meyer *et al.*, 1982; Persoone *et al.*, 1980). The test samples were then applied against *Artemia salina* in a 1-day *in vitro* assay. The experiments were carried out in triplicate using vincristine sulphate as standard and the results have been shown as mean \pm SEM (standard error of mean).

The antioxidant activity (free radical scavenging activity) of the extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined by following standard protocol developed by Brand-Williams (Wichi,

1988; Auddy *et al.*, 2003). Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated by using *tert*-butyl-1-hydroxyl toluene (BHT), a potential antioxidant as positive control. The experiments were carried out in triplicate and the results have been shown as mean \pm SEM (standard error of mean).

The primary data obtained from the experiments were manipulated as the source of responses. For each of the extracts, three samples were prepared for each of the bioassay. Data were expressed as mean \pm SEM (standard

error of mean). Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $p < 0.05$.

Different partitionates of methanol extract of *G. globosa* were tested for antibacterial and antifungal activities against a number of gram positive and gram negative bacteria as well as some fungi. Among all the partitionates, the carbon tetrachloride and chloroform soluble fraction of the methanol extract exhibited mild to moderate antibacterial and antifungal activity (Table 1).

Table 1. Antimicrobial activity of *G. globosa* extractives at 400 μ g/ml per disc

Test organisms	Diameter of the zone of inhibition (mm)			
	CME	CTSF	CSF	Ciprofloxacin
Gram positive bacteria				
<i>Bacillus cereus</i>	7 \pm 0.48	9 \pm 0.100	11 \pm 0.233	44 \pm 0.234
<i>B. megaterium</i>	6.5 \pm 0.23	8 \pm 0.208	10 \pm 0.345	44 \pm 0.33
<i>B. subtilis</i>	6.8 \pm 0.55	9 \pm 0.401	11 \pm 0.221	44 \pm 0.200
<i>Staphylococcus aureus</i>	5 \pm 0.025	9 \pm 0.115	11 \pm 0.337	44 \pm 0.370
<i>Sarcina lutea</i>	7.4 \pm 0.67	10 \pm 0.260	9 \pm 0.342	44 \pm 0.33
Gram negative bacteria				
<i>Escherichia coli</i>	5 \pm 0.43	9 \pm 0	12 \pm 0.273	44 \pm 0
<i>Pseudomonas aeruginosa</i>	6 \pm 0.50	9 \pm 0.045	14 \pm 0.069	44 \pm 0.127
<i>Salmonella typhi</i>	5.5 \pm 0.56	8 \pm 0.076	10 \pm 0.180	44 \pm 0.100
<i>S. paratyphi</i>	7.6 \pm 0.098	9 \pm 0.197	11 \pm 0.120	44 \pm 0.402
<i>Shigella boydii</i>	7.4 \pm 0.65	10 \pm 0.208	9 \pm 0	44 \pm 0
<i>Sh. dysenteriae</i>	5.8 \pm 0.47	9 \pm 0.445	11 \pm 0.227	44 \pm 0.450
<i>Vibrio mimicus</i>	7 \pm 0.32	11 \pm 0.09	9 \pm 0	44 \pm 0.05
<i>Vibrio parahaemolyticus</i>	5 \pm 0.25	9 \pm 0	12 \pm 0.443	44 \pm 0
Fungi				Kanamycin
<i>Candida albicans</i>	7 \pm 0.40	9 \pm 0.173	13 \pm 0.34	25 \pm 0.167
<i>Aspergillus niger</i>	6.5 \pm 0.45	10 \pm 0.167	11 \pm 0.260	25 \pm 0.33
<i>Saccharomyces cerevisiae</i>	6 \pm 0.43	9 \pm 0.303	8 \pm 0	25 \pm 0

CME= Crude methanol extract; CTSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction

Table 2 shows the results of the brine shrimp lethality assay after 24 hr exposure to the samples and the positive control vincristine sulfate. The positive control, compared with the negative control (DMSO) was lethal, depicting significant mortality to the shrimp.

The median lethal concentration (LC_{50}) of the test samples after 24 hr was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the graph by means of regression analysis. Among all the fractions of crude methanol extract of *Gomphrena globosa* (L.), the chloroform soluble fraction exhibited highest lethality having LC_{50} value (0.331 \pm 0.029) μ g/ml.

Table 2. Results of cytotoxicity screening of *G. globosa*

Test Samples	Regression line	R ²	LC ₅₀ (μ g/ml)
CTSF	Y= 42.28x – 0.739	0.957	15.851 \pm 0.148
CSF	Y= 18.52x + 58.89	0.929	0.331 \pm 0.029
VS	Y= 30.8x + 60.64	0.972	0.451 \pm 0.0207

VS= Vincristine sulfate, CTSF= Carbon tetrachloride soluble fraction, CSF= Chloroform soluble fraction, LC_{50} (μ g/ml) values of different fractions are expressed as mean \pm SEM (n=3); SEM: standard error of mean

Table 3 shows that among the entire fractions of *G. globosa*, the crude methanol extract exhibited significant and its hexane soluble fraction showed highest free radical scavenging activity having IC_{50} values (20.35 \pm 0.360) μ g/ml and (13.17 \pm 0.308) μ g/ml respectively.

Table 3. IC₅₀ values of standard and partitionates of *G. globosa* in DPPH assay

Sample	IC ₅₀ (µg/ml)
CME	20.35±0.360
NHSF	13.17±0.308
BHT	20.39±0.245

CME= crude methanolic extract, NHSF=n-Hexane soluble fraction, BHT= tert-butyl-1-hydroxytoluene

On the basis of the above biological screenings and folk medicinal uses it can be concluded that this plant has significant medicinal properties. It is also clearly evident from the above findings that the whole plant of *G. globosa* has high antioxidant potential and significant cytotoxic activities. Therefore, the plant is a good candidate for further studies to isolate the bioactive principles.

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