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Investigation of Biological Properties of *Gomphrena globosa (L.),* Family: Amaranthaceae

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ABSTRACT

The plant *Gomphrenaglobosa* (L.) is an important medicinal plant of Bangladesh and used for oliguria, heat & empacho, hypertension, cough & diabetes, expectorant for animals and many others medicinal purposes locally. In order to observe different traditional uses in laboratory, crude methanolic fraction, n-Hexane soluble fraction, carbon tetrachloride soluble fraction, chloroform soluble fraction & aqueous soluble fraction of whole plant of *Gomphrenaglobosa* (L.) were subjected to antioxidant, brine shrimp lethality bioassay & antimicrobial screening tests. Among the entire fractions n-Hexane soluble fraction exhibited the highest antioxidant activity having IC_{50} value (13.17±0.308) µg/ml & highest phenolic content with (57.12±0.265) mg of GAE / gm of extractives and crude methanolic fraction showed significant antioxidant activity with IC_{50} value (20.35±0.360) µg/ml & (41.02±0.49) mg of GAE / gm of extractives which represents a positive correlation between free radical scavenging activity and total phenolic content. Additionally chloroform soluble fraction showed significant cytotoxicity having LC_{50}(0.331±0.029) µg/ml and carbon tetrachloride soluble fraction & chloroform soluble fraction exhibited mild to moderate antimicrobial activity having zone of inhibition (8±0.208)to (14±0.069) mm.

Key words: *Gomphrenaglobosa* (L.), antimicrobial activity, brine shrimp lethality bioassay, antioxidant activity, total phenolic content.

INTRODUCTION

Gomphrena globosa (L.), Bengali name-Botamphul.Family- Amaranthaceae is an annual branched herb, cultivated as ornamental flowering herb in garden [1]. It is native to North-America, South-America, Myanmar, and Indiaand well grows over Bangladesh which is commonly known as globe amaranth. Its Leaves 5 -10 cm, subsessile, elliptic or obovate-oblong, acute or obtuse. Heads 2.5-3.7 cm diam., pinkish purple, globose with 2 leafy bracts, peduncled, terminating the branches. An annual plant having 1to2 feet height, spreading 1to5 feet with round densed and slow growing rate.Amaranthaceae is a large family comprising around 10 sub families, 176 genera and 2400 species available all over the world [2, 3]. Gomphrena globosa (L.) is a folk remedy for oliguria, heat &empacho, hypertension [4], cough & diabetes [5] and expectorant for animals [6]. Since this plant has important medicinal properties, present study has been undertaken to observe various medicinal properties in laboratory. Herein, the study reports, for the first time, antioxidant property, brine shrimp lethality bioassay and antimicrobial screening of the whole plant, Gomphrenaglobosa (L.).

MATERIALS & METHODS

Collection of Plant material

Whole plant of Gomphrenaglobosa (L.) was collected from Dhaka in November 2011. This plant was identified by the taxonomist of the Botany Department of the University of Dhaka. A voucher specimen (Accession no. 3557) for this collection has been deposited in University of Dhaka Herbarium for future reference.

Extraction and partitioning

The powdered whole plant (800 g) of Gomphrena globosa (L.) 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 into n-hexane, carbon tetrachloride, and chloroform [7]. Subsequent evaporation of solvents afforded n-hexane (2 g), carbon tetrachloride (1.75 g), chloroform (0.5 g) and aqueous soluble (0.75 g) materials. Partitionates obtained by this way were subjected to different biological screening tests.

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Antimicrobial screening

The antimicrobialactivity of the extractives was determined by the discdiffusion method [8, 9, 10, 11].

5 gram positive bacterial, 8 gram negative bacterial and 3 fungal strains used for the experiment (Table 1) were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The extractives were dissolved separately in chloroform and methanol as required and applied to sterile filter paper discs at 400 µg/disc and carefully dried to evaporate the residual solvent. Standard ciprofloxacin (5µg/disc) discs were used as positive control for both gram positive & gram negative bacteria and standard kanamycin (30µg/disc) used as positive control for fungi. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiments were carried out in triplicate and the results have been shown as mean ± SEM (standard error of mean).

Brine shrimp lethality bioassay

For cytotoxicity screening, the n-hexane, carbon tetrachloride and chloroform soluble materials of crude methanolic extract were separately dissolved in DMSO [12, 13, 14]. The test samples were then applied against Artemia salina in a 1-day in vitro assay. Artificial sea water was prepared as described by Culkin with slight modification of chemical composition. Four mg of each of the extractives was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 µg/mL were obtained by serial dilution technique. Vincristine sulfate and DMSO were used as the positive control and negative control respectively. The median lethal concentration (LC50) of the test samples

after 24 hrs of exposure were determined from a plot of % of mortality of shrimps against the logarithm of the sample concentration. The experiments were carried out in triplicate and the results have been shown as mean ± SEM (standard error of mean).

Antioxidant evaluation

DPPH assay

The antioxidant activity (free radical scavenging activity) of the extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Brand-Williams [15, 16, 17]. In the experiment, 2.0 mg of each of the extract was dissolved in methanol. Solution of varying concentrations such as 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml were obtained by serial dilution technique. 2 ml of a methanol solution of the extract of each concentration was mixed with 3 ml of a DPPH-methanol solution (20 µg/ml) and was allowed to stand for 20 minutes for the reaction to occur. Then the absorbance was determined at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation:

% inhibition = [1- (Abssample/ Abscontrol)] x 100

Then % inhibitions were plotted against respective concentrations used and from the graph IC50 was calculated by using tert-butyl-1-hydroxytoluene (BHT), a potential antioxidant, was used as positive control. The experiments were carried out in triplicate and the results have been shown as mean ± SEM (standard error of mean).

Total phenolic content (TPC)

To 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from gallic acid solution with different concentration, the total phenols content of the sample was measured [18, 19] .The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extract. The experiments were carried out in triplicate and the results have been shown as mean ± SEM (standard error of mean).

Statistical analysis

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 ± 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 $p < 0.5$.

RESULT

Antimicrobial activities

Different partitionates of methanol extract of *Gomphrena globosa* (L.) 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 soluble & chloroform soluble fraction of the methanol extract exhibited mild to moderate antibacterial and antifungal activity (Table1). Carbon tetrachloride soluble fraction showed zone of inhibition (8±0.208) to (10±0.260) mm and chloroform soluble fraction showed (9±0.342) to (11±0.233) mm zone of inhibition against gram positive bacteria; (9±0) to (11±0.09) mm &(9±0) to (14±0.069) mm respectively for gram negative bacteria in comparing with standard ciprofloxacin that possesses zone of inhibition (44±0) mm.

Table1. Antimicrobial activity of carbon tetrachloride soluble fraction (CTSF) & chloroform soluble fraction (CSF) of *Gomphrenaglobosa* (L.) at 400µg/ml per disc.*

Test organisms	Diameter of the zone of inhibition (mm) CTSF	CSF	Ciprofloxacin
Gram positive bacteria			
Bacillus cereus	9±0.100	11±0.233	44±0.234
Bacillus megaterium	8±0.208	10±0.345	44±0.33
Bacillus subtilis	9±0.401	11±0.221	44±0.200
Staphylococcus aureus	9±0.115	11±0.337	44±0.370
Sarcinalutea	10±0.260	9±0.342	44±0.33
Gram negative bacteria			
Escherichia coli	9±0	12±0.273	44±0
Pseudomonas aureus	9±0.045	14±0.069	44±0.127
Salmonella paratyphi	9±0.197	11±0.120	44±0.402
Salmonella typhi	8±0.076	10±0.180	44±0.100
Shigellaboydii	10±0.208	9±0	44±0
Shigelladysenteriae	9±0.445	11±0.227	44±0.450
Vibrio mimicus	11±0.09	9±0	44±0.05
Vibrio parahemolyticus	9±0	12±0.443	44±0
Fungi			Kanamycin
Candida albicans	9±0.173	13±0.34	25±0.167
Aspergillusniger	10±0.167	11±0.260	25±0.33
Sacharomycescerevacae	9±0.303	8±0	25±0

**The diameters of zone of inhibition are expressed as mean ± SEM (n=3); SEM: standard error of mean.*

Table 2. Results of cytotoxicity screening of *Gomphrena globosa* (L.).**

Test samples	Regression line	R^2	LC_{50} (µg/ml)
CTSF	Y=42.28x-0.739	0.957	15.851±0.148
CSF	Y=18.52x+58.89	0.929	0.331±0.029
VS	Y=30.8x+60.64	0.972	0.451±0.207

***VS= vincristine sulfate, CTSF= Carbon tetrachloride soluble fraction, CSF= Chloroform soluble fraction, LC_{50} (µg/ml) values of different fractions are expressed as mean ± SEM (n=3); SEM: standard error of mean.*

Table 3. IC_{50} values of the standard and partitionates of *Gomphrenaglobosa* (L.) in DPPH assay.***

Sample	IC_{50} (µg/ml)
CMF	20.35±0.360
NHSF	13.17±0.308
BHT	20.39±0.245

****CMF= crude methanolic fraction, NHSF=n-Hexane soluble fraction, BHT= tert-butyl-1-hydroxytoluene, IC_{50} (µg/ml) values of different fractions are expressed as mean ± SEM (n=3); SEM: standard error of mean.*

Table 4: Total phenolic content of NHSF & CMF of *Gomphrena globosa* (L.).****

Sample code	Total phenolic content (mg of GAE/gm of extractives)
NHSF	57.12±0.265
CMF	41.02±0.49

*****GAE= Gallic acid equivalent, CMF= crude methanolic fraction, NHSF=n-Hexane soluble fraction, total phenolic content of different fractions is expressed as mean ± SEM (n=3); SEM: standard error of mean.*

On the contrary, carbon tetrachloride soluble fraction exhibited (9±0.173) to (10±167) mm & chloroform soluble fraction showed (8±0) to (13±0.34) mm zone of inhibition comparing with standard kanamycin that possesses zone of inhibition (25±0) mm.

Cytotoxic activities

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 methanolic extract of *Gomphrena globosa* (L.), chloroform soluble fraction exhibited highest lethality having LC_{50}value (0.331±0.029)µg/ml.

In vitro Antioxidant activity

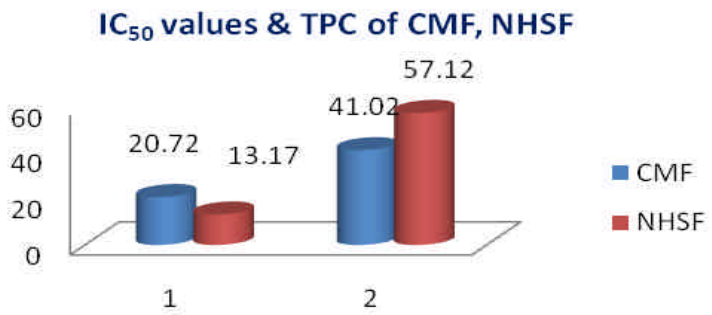
DPPH assay

Table 3 shows that among the entire fractions of *Gomphrenaglobosa* (L.) crude methanolic fraction exhibited significant and chloroform soluble fraction showed highest free radical scavenging activity having IC_{50} values (20.35±0.360) µg/ml & (13.17±0.308) µg/ml respectively.

Total phenolic content (TPC)

Table 4 showed the highest phenolic content for *Gomphrena globosa* (L.) was found in n-Hexane soluble fraction (NHSF), (57.12±0.265 mg of GAE / gm of extractives) followed by crude methanolic fraction (41.02±0.49 mg of GAE / gm of extractives).

Correlation between IC_{50} values & TPC of *Gomphrena globosa* (L.)



DISCUSSION

The current study established that the various fractions of the whole plant of *Gomphrena globosa* (L.) showed strong anti-oxidant activity & presence of significant amount of phenolic compounds which bridges a positive correlation that the anti-oxidant activities of plant extracts are mainly attributed to the presence of phenolic compounds. The slight anti-microbial activity present in the carbon-tetrachloride soluble fraction and the chloroform soluble fraction may be caused by the disruption of the cell membrane or may be due to the inhibition of protein synthesis. The strong cyto-toxic activities present in the chloroform soluble fraction may be due to the presence of cyto-toxic compounds. Therefore further study may be recommended to find out that the anti-cancer potential of the plant.

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