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ANTIOXIDANT POTENTIAL OF LEAVES OF *GOMPHHRENA GLOBOSA*

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ABSTRACT
Crude methanol extract and its n-butanol, petroleum ether and ethyl acetate fractions of *Gomphhrena globosa* Linn. (Amarnthaceae) leaves were screened for antioxidant potential by1,1-Diphenyl-2-picrylhydroxyl (DPPH) assay, 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) assay and nitric oxide assay. Total phenolic and flavanoids contents of different fractions were also determined. n-Butanol fraction showed significant antioxidant potential compared to other fractions. The total phenolic and flavonoid content in n-butanol fractions was 16.10 ± 0.25 mg gallic acid equivalent (GAE)/g extract and 12.15 ± 0.15 mg/quercetin/g extract was significant when compared with other fractions.

KEYWORDS: Antioxidant potential, Fractions, Total phenolics, total flavonoids.

INTRODUCTION
Antioxidants are substances that significantly delay or inhibit oxidation of an oxidizable substrate.[1] Free radicals causes aging, destruction of DNA, obstruction of arteries, obesity, cancer, strokes, cardiac and central nervous system (CNS) disorders, which have led to an increase in the investigation of substances that can protect against these reactive oxygen species and thus may play a role in disease prevention.[2,3] Exogenous antioxidants which are derived from sources outside the living systems such as diets[4] stimulate cell repair externally.

Gomphhrena globosa Linn. (Amarnthaceae), is ornamental plant popularly known in Kannada as Nelli Rudrakshi. Its leaves and flowers traditionally used in the treatment of hypertension, diabetes, kidney problems[5], cough, bronchitis and other respiratory diseases mainly due to expectorant properties. Besides as an ornamental plant it is commonly used for the treatment of jaundice, high cholesterol and urinary problems in Latin America and Caribbean as abortiacient in South America.[6,7] Whole plant decoction is applied to gangrenous wound. In South America, the plant is utilized as an abortifacient and decoction of the whole plant of *Gomphrena globosa* is applied to gangrenous wound.[8] Phytochemical present in *Gomphrene globosa:* betacyanins, β-sitosterol, flavonoids, stigmastreol and hydroxycinmamides.[9,10]

MATERIALS AND METHODS
Preparation of extracts
Leaves of **Gomphhrena *Globosa*** were dried under shade and extracted with methanol in a Soxhlet apparatus for 72 h at 68 ºC. The extract was dried to a dark brown sticky mass in a rotary evaporator under controlled temperature and pressure. The methanol extract (GGM) was suspended in distilled water and partitioned successively with petroleum ether (60-80 ºC) (GGPE), ethyl acetate (GGEA), n-butanol (GGBU).

Determination of total phenolic content[11]
Using Folin–Ciocalteu method, total phenol contents in the extracts were determined. An aliquots of 1ml of extracts or standard solution of Gallic acid (2, 4, 6, 8,10 µg/ml) was added to a 25ml volumetric flask, containing 9ml of distilled water. 1 ml of Folin-ciocalteu reagent was added to the mixture and shaken. After 5min, 10ml of 7% Na_2CO_3 was added to the mixture. The solution was made up the mark to 25ml with distilled water and mixed. After incubation for 90min at room temperature, the absorbance against the prepared reagent blank was determined at 750nm with an UV-Visible spectrophotometer (Shimadzu). All Samples were analysed in duplicates.

Determination of Total flavonoid content[12]
Estimation of the total flavonoids in the plant extracts was carried out using the aluminium chloride colorimetric assay. An aliquots of 1ml of extracts or standard solution of Quercetin (2, 4, 6, 8,10 µg/ml) was added to a 10ml volumetric flask containing 4ml of

water. To the above mixture, 0.3ml of 5% NaNo2 was added. After 5min, 0.3ml of 10% AlCl3 was added. After 6min, 2ml of 1 M NaoH was added and the total volume was made up to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepapred reagent blank at 510 nm.

IN-VITRO ANTIOXIDANT ACTIVITY

DPPH radical scavenging assay[13]

This test was measured as described by Blois. DPPH was dissolved in methanol to a 0.025 g/L. The plant extract at various concentrations was diluted with dimethyl sulfoxide (DMSO) to get sample solution. 5 µL of the sample solution in a 96-well plate following which 195 µL DPPH working solution was added to each well. After a 20 min reaction at room temperature, the absorbance of the solution was measured at 515 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation:

DPPH scavenging activity (%)= $(A_0-A_1)/ A_0x100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

ABTS scavenging activity[14]

ABTS radical-scavenging activity of the extract was determined according to method described by Re et al. (1999). The free ABTS radical was generated in the solution by reacting 7 mM ABTS solution and 2.45 mM potassium persulfate. This mixture was incubated in dark for 15 hr and diluted with methanol to obtain an absorbance of 1.1±0.2 units at 750 nm. Various concentrations of plant extract/fractions were prepared in methanol and 20 µL of extract solution was added to 180 µL of ABTS free radical cation solution. The reaction mixture was incubated for 20 min and absorbance read at 750 nm. All samples were assayed in triplicate. Ascorbic acid was used as a positive control.

ABTS scavenging activity (%)= $(A_0-A_1)/ A_0x100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Nitric oxide radical scavenging activity assay[15]

The nitric oxide scavenging activity can be assessed by the Griess reaction assay with some modifications. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable nitrate and nitrites. The quantities of which can be determined using griess reagent. The reaction mixture consisted of 2 mL 10 mM sodium nitroprusside, 0.5 mL phosphate buffered saline and 0.5 mL of various concentrations of extract/fractions. The mixture was incubated for 150 min at 25 ºC and 0.5 mL of the mixture was mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid). It was allowed to stand for 5 min followed by addition of 1 mL of 1% naphthyl ethylenediamine dihydrochloride. The mixture was incubated for 30 min and absorbance measured at 540 nm against corresponding blank. Curcumin was used as positive control. All samples were assayed in triplicate.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and the differences between samples were determined by Graph Pad Prism software version 5.0.

RESULTS AND DISCUSSION

The total phenolic contents is the highest in n-butanol fraction of *G. globosa* was 16.10± 0.25 mg and least in methanol extract 9.40 ± 0.11mg GAE/g respectively. The total phenol content was in the following order: GGBU>GGPE>GGEA>GGM (Table 1).

The highest content of flavonoid was found to be in GGBU (12.15 ± 0.15) and higher than the other fractions. The total flavonoid content was in the following order: GGBU>GGM>GGEA>GGPE **(**Table 1).

DPPH is a stable, free radical by virtue of the delocalization of the spare electron over the molecule as a whole. When a solution of DPPH is mixed with the substrate that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet color. The extract/ fractions showed concentration-dependent DPPH scavenging activity. (Table No.2).

Nitric oxide plays an important role in various inflammatory processes in the body and their toxicity multiplies only when they react with $O_2^{\cdot-}$ to form peroxynitrite which causes further damage to cellular biomolecules like proteins, lipids and nucleic acids. The extract and fractions of *Gomphhrena Globosa* inhibited nitrite formation by competing with oxygen to react with nitric oxide formed by the reaction of sodium nitroprusside in physiological solution in a concentration-dependent manner. The potency of the extract/fractions was found to be in the following order: GGBU>GGM>GGEA>GGPE. (Table No.3).

The ABTS free radical was generated by the reaction of ABTS with potassium persulfate to generate a blue-green $ABTS^{\cdot+}$ chromophore. The ability of extract solutions to decolorize the chromophore was measured as percentage scavenging of ABTS radicals. ABTS free radical scavenging increased with increasing concentrations of fractions. The potency of the extract/fractions was found to be in the following order: GGBU>GGM>GGEA>GGPE. (Table No.4).

Plants have diverse groups of phenolic compounds, such as simple phenolics, phenolic acids, anthocyanins,

hydroxycinnamic acid derivatives and flavonoids. All these phenolic classes have gained extensive attention because of their physiological functions, including free radical scavenging, anti-mutagenic, anticarcinogenic and anti-inflammatory effects.[16] According to Pietta[17], the antioxidant activity of phenolics is largely due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

Table 1: Total polyphenol and Flavonoid content.

Extract/Fraction	Total Polyphenol content (mg/g)	Total Flavonoid content (mg/g)
GGM	9.40 ± 0.11	8.34 ± 0.10
GGPE	13± 0.18	3.10 ± 0.28
GGBU	16.10± 0.25	12.15± 0.05
GGEA	10± 0.15	7.27 ± 0.05

Table 2: Free radical scavenging activity by DPPH method.

% absorbance by Fractions				
Concentration (µg/mL)	GGM	GGPE	GGBU	GGEA
10	16.01	8.13	18.16	10.22
20	21.53	11.12	23.02	18.06
30	29.02	21.09	31.23	24.19
40	35.13	31.00	44.08	32.01
50	42.04	20.02	59.16	38.18

Table 3: ABTS scavenging activity.

% absorbance by Fractions				
Concentration (µg/mL)	GGM	GGPE	GGBU	GGEA
10	16.01	8.13	18.16	10.22
20	21.53	11.12	23.02	18.06
30	29.02	21.09	31.23	24.19
40	35.13	31.00	44.08	32.01
50	42.04	20.02	59.16	38.18

Table 4: Nitric oxide scavenging activity.

% absorbance by Fractions				
Concentration (µg/mL)	GGM	GGPE	GGBU	GGEA
10	28.07	19.16	38.01	12.18
20	31.13	27.21	43.14	21.09
30	42.02	32.14	51.02	29.02
40	56.05	41.05	69.23	39.05
50	61.08	58.29	74.12	47.27

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