ACADEMICIA
An International Multidisciplinary Research Journal
(Double Blind Refereed & Peer Reviewed Journal)

DOI: 10.5958/2249-7137.2021.02347.8

GOMPHERNA GLOBOSA: THE POTENTIAL NATURAL FOOD GRADE BETACYANIN

Lavanya V*; Thamaraiselvi SP**

*Doctoral Student,
Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu, INDIA

**Assistant Professor,
Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu, INDIA
Email id: twinklelavan@gmail.com

ABSTRACT

Betalain are natural pigments and highly water soluble nitrogenous compounds which are commonly found in the order Caryophyllales and in some fungi species. Considering the significance of natural colourants in the food industry, the present study aimed to bring the benefits and potentiality of Gompherna globosa into limelight as a food dye. The pigment has been extracted using aqueous solution as a solvent and they are quantified by Spectroscopic method and HPLC method and the extracts were characterized for higher Anti-oxidant, Anti-microbial. The storage effects of different temperature and light intensities on the Gompherna extract were measured where maximum degradation was observed when the pigment was stored under room temperature and under light on 14^{th} and 7^{th} day of storage. On concerning the stability of Gompherna extract, the lyophilized extract was used as a dye by incorporating in Ice-cream and the dried flower was used for making tea. The betalain flavoured ice-cream and Gompherna tea were preferred more by the active respondents compared to the plain vanilla Ice-cream and black tea for the appealing colour, Texture, Taste and palatability.

KEYWORDS: *Gompherna Globosa, Quantification, Anti-Oxidant, Anti-Microbial, Stability and Food Dye.*

1. INTRODUCTION

Colour is a major character of flowers which not only add attraction,elegance and beauty but also serve as a sources for natural pigments which in turn used in colouring of food products and textiles.

Synthetic dyes and pigments are the major pollutants affecting the environment, soil and water resources and thereby cause health issues to the humans. To overcome the harmfulness caused by the synthetic dyes to humans and environment, researchers have involved in the development of natural dyes and pigment (Kumar *et al.*, 2017).

Natural pigments are less stable due to different factors and are higher in cost of extraction compared to synthetic dyes and hence more attention is needed in their utilization and development. (Sabarudin *et al.*, 2016).

Betalains were one of the natural pigment found in Plants which is a potential food dye giving a wide range of colour from red to Violet. Betalains are highly water soluble nitrogenous compounds, commonly found in the order Caryophyllales and in some fungi species. Betalains are predominantly found in Beetroot, Opuntia, Bougainvillea, Gomphrena, Celosia etc.

Previous studies had stated that betacyanin was extracted from fruits of cactus (*Opuntiaficus-indica*), dragon fruit (*Hylocereuspolyrhizus*) and red beet (*Beta vulgaris L. ssp. Vulgaris*) which is widely used as a food colourant in many dairy products, beverages, candies and cattle products that give vivid red colour. Apart from the use as a colourant, betalains have a wide range of biological activities with potential health benefits like they counter inflammation, protect the liver and have anticancer, antitumor and antioxidant properties.

Gomphrena species is an edible, Ornamental and medicinal plant commonly known as Globe Amaranth or Bachelor Button, belongs to the family Amaranthaceae. Though extraction procedures for betalains have been reported in crops like, red beet, and *Opuntia*, research on extraction of betalain pigments from flowers is meager and needs to be exploited. Considering the importance of flowers as sources of natural betalain pigments, the present paper aimed to extract, quantify and expose the antioxidant, antimicrobial propertiesand stability of betalains from *Gomphrena globosa* and their potential usage as a food dye.

2. MATERIALS AND METHODS

2.1 Sample preparation

One gram of shade dried Gompherna petals were macerated with 100ml of solvent (HPLC grade distilled water) and kept in a shaker overnight for incubation. Then the pigments were extracted by filtering the solution in a Whattman no.30 filter paper and the filtrate was stored under -20°C for further analysis.

2.2 Estimation of total betalain content

The aqueous extracted pigment was diluted using McIlvaine buffer (pH 6.5, citrate-phosphate buffer). The absorption was measured at different OD values at 538, 480, and 600nm for the quantification of betacyanin, betaxanthin, and total betalains respectively using UV-VIS spectrophotometer (Eppendorf bio spectrometer) (Moßhammer *et al.*, 2005)

The betalain content (BC) was analyzed by the following formula,

$$\text{Betalain Content}(mg/l) = \frac{A * DF * MW * 1000}{\epsilon * l}$$

Here,

A -Absorption value at 600 nm

DF - dilution factor

l -Path length (1 cm) of the cuvette

For quantification of betacyanins and total betalain- the molecular weights (MW) = 550 g/mol and molar extinction coefficients (ε) = 60 000 L/ (mol cm) in H_2O; λ=538 nm for betacyanin and λ=600 for total betalain.

For quantification of betaxanthins -the molecular weights (MW) = 308 g/mol; and molar extinction coefficients (ε) = 48 000 L/ (mol cm) in H_2O; λ=480 nm

2.3 Lyophilization

The aqueous extracted sample was concentrated for betalain pigments using lyophilizer (Lark, Penguin classic). Freeze drying at -80°C temperature and 0.1mPa pressure yielded freeze dried powder of the Gompherna betalain extract and were stored in vials at deep freezer(-80°C) to prevent from degradation.

2.4 Quantification by HPLC

Betanin was quantified by water modular liquid chromatographic system (Shimadzu LC- 88 A) equipped with two M510 pumps, a M996 photodiode array detector and a rheodyne model 7125 injector and a sample loop of 20µl was used, along with a Millenium 2010 chromatography data management system. A kromasil 100 $C_{18,}$ 5µM, 25 cm**x** 4.6 mm I.D column was used and elution was carried out following a modification of the chromatographic program proposed by (Fernández-López *et al.*, 2002). The program consisted of two mobile phase solvent A (1% acetic acid in water) and solvent B (1% acetic acid in acetonitrile) with a flow rate of 1ml/min.

2.4.1 Preparation of betanin standard

The betanin standard was diluted with HPLC grade distilled water since betalain is highly soluble in water. Freshly prepared standard was used for analysis. The concentration of the standard used for HPLC was 100ppm.

2.4.2 Preparation of Gompherna extracts for HPLC quantification

The Gompherna extract was prepared by dissolving the freeze dried sample in HPLC grade distilled water. For each analysis, 20µl of the filtered extract was directly injected into the chromatographic column. The identities of the different chromatographic peaks were confirmed by their visible spectral characteristics in comparison with the standards and their retention times.

2.5 Assessment of antioxidant activity of Gompherna betalain extract

The antioxidant activity of Gompherna extract was analyzed by two major methods namely, Free radical scavenging activity and TotalReducing power assay. For the estimation of antioxidant, the Gompherna extract was prepared at different concentrations using distilled water viz., 1250, 1000, 750, 500, 250 µg/ml.

2.5.1 Free radical scavenging activity

2.5.1.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) method

DPPH is a stable free radical which do not dimerize as other free radicals. Occurrence of purple colour indicates the delocalization of DPPH molecule, with an absorption of around 517nm. The betalain extract was measured for antioxidant activity by its ability to scavenge the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) (Wong *et al.*, 2006). Hundred micro litre of the prepared extract was treated with three ml of a DPPH reagent having absorbance of 0.980±0.02 in methanol initially and incubated at normal ambient temperature. After 20 min of incubation, the absorbance was measured at 517nm using UV/Vis spectrophotometer.

2.5.1.2 ABTS (2, 2'-azino-bis (3- ethylbenzthiazoline-6-sulphonic acid) method:

The oxidation of ABTS cation radical is determined by the loss of electron in a nitrogen atom which is physically indicated by the presence of bluish green color. 300 µl of the extract was mixed with 3 ml of 7mM ABTS and 2.45mM potassium persulphatereagent having absorbance of 0.7±0.05 in methanol at 743 nm and incubated at normal ambient temperature. After 6 min of incubation, the absorbance was measured at 743 nm using UV/Vis spectrophotometer.

Ascorbic acid at different concentrations (1 mg/ml to 5 mg/ml)was used as standard for both the methods. The percentage of inhibition was calculated by the below formula,

$$\% \text{ inhibition} = \frac{(\text{Initial absorbance} - \text{final absorbance})}{\text{Initial absorbance}} * 100$$

The concentration required for 50% reduction of ABTS (IC_{50}) and DPPH (IC_{50}) was used to express the antioxidant capacity of the samples (Yıldız *et al.*, 2008)

2.5.2 Total reducing power assay

2.5.2.1 FRAP (Ferric ion reducing antioxidant power) method

The FRAP reagent was prepared by adding 25ml of 300 mM Acetate buffer maintained at a pH of 3.6 to which 2.5 ml of 10mM TPTZ (2, 4, 6- tri (2-pyridyl)-s-triazine) was dissolved in 40 mM HCL and added with 2.5 ml of 2.mM $FeCl_3$.100µl of the extract was added to 1.9ml of FRAP reagent and the reaction mixture was incubated for 30 minutes under water bath and maintained at 36°C. The ferric ions get reduced after adding the extract indicating the antioxidant potential of the extract which was measured after incubation under UV/Vis spectrophotometer at 593nm. The Ferric reducing antioxidant potential of the flower extracts was expressed as µM Ascorbic acid equivalent (Brand-Williams *et al.*, 1995)

2.5.2.2 CUPRAC (Cupric reducing antioxidant power) method

CUPRAC reagent was prepared by mixing together 1mL of 1.0×10^{-2}M copper (II) chloride with 1mL of 1M ammonium acetate buffer maintained at pH 7.0, at which the reaction mixture was

added with 1mL of 7.5×10^{-3}M neocuproine solution. Hundred microlitre of the flower extract was added to 1ml of CUPRAC reagent. The reaction mixture was then incubated at room temperature for 30 minutes and their absorbance was recorded at 450nm using UV/Vis spectrophotometer. (Sahreen *et al.*, 2010) A standard calibration curve was developed using ascorbic acid and CUPRAC reagent. The cupric ion reducing antioxidant capacity of the flower extract was expressed as µM Ascorbic acid equivalent.

2.5.2.3 Chelating potential

The extracts were dissolved in water to prepare various sample solutions at 1250, 1000, 750, 500, 250 µg/ml. To 200µl of the sample prepared, 100µl of $FeCl_2.2H_2O$ (2.0 mM) and 900µl of methanol were added. After 5 min of incubation under room temperature, 5.0 mM of 400 µl of ferrozine is added. The absorbance was measured after 10 min at 562 nm using UV/Vis spectrophotometer

The chelating activity (%) was calculated using the following equation,

$$\% \text{ inhibition} = \frac{(\text{Initial absorbance} - \text{final absorbance})}{\text{Initial absorbance}} * 100$$

Ascorbic acid was used as a standard. The concentration required for the 50% reduction of the chelates (IC_{50}) was used to express the antioxidant capacity of the samples. (Karawita *et al.*, 2005).

2.6 Assessment of antimicrobial properties of betalain extracts

2.6.1 Preparation of betalain extract

Lyophilized *Gomphrena globosa*extract was dissolved in distilled water to get different concentrations as follows,

Treatments	**Concentration of samples for inoculation**
T_1	100 mg/ml
T_2	200 mg/ml
T_3	300 mg/ml
T_4	400 mg/ml
T_5	500 mg/ml

2.6.2 Preparation of culture media

Common food borne species of bacteria and fungi (obtained from the Department of Microbiology, TNAU) were cultured and maintained on nutrient agar (NA) medium (Allen, 1953) and Potato dextrose agar (PDA) medium (Sabouraud, 1892). For the analysis of antimicrobial activity by, a loop of the organism *viz*., bacteria and fungi was inoculated into 100 ml of the nutrient broth and potato dextrose broth respectively. The conical flasks were incubated at a temperature of 37°C for 24-48 h for bacteria and 3-6 days for fungi.

The antimicrobial activity of the Gompherna extract was analyzed by Agar well diffusion method with a Each well was loaded with 20µl of the prepared flower extract at different concentration using sterilized pipette with two replications maintained with a positive control

(antibacterial agent Ampicillin and antifungal agent cyclohexamide at a concentration of 10 mg/ml) and negative control as distilled water.

After an incubation period of 24 hours for bacteria and 3 days for fungi, the observations were recorded by measuring the inhibition zone (clearing zone), which indicate the absence of microbial growth around the well. The diameter of inhibition zone (Yıldız *et al.*) was measured and the mean D1Z was calculated. The antimicrobial activity was assessed by calculating the relative inhibition zone diameter (RIZD).

$$\text{RIZD (per cent)} = \frac{\text{DIZ of sample - DIZ of negative control}}{\text{DIZ of positive control}} \times 100$$

2.6.3 Microorganisms tested

	Bacteria	
1.	*Escherichia coli*(O157 strain)	Gram - ve
2.	*Pseudomonas aeruginosa*	Gram - ve
3.	*Bacillus subtilis*	Gram - ve
	Fungi	
1.	*Rhizopus spp*	
2.	*Aspergillus niger*	

2.7 Stability of betalain extract of Gompherna at different temperatures

The effect of different storage temperature on betalain stability was analyzed. The concentrated extracts were taken in screw-capped vials and stored at different temperatures as follows,

Treatments	**Temperature levels**
T_1	- 80^oC (deep freezer)
T_2	-20^oC
T_3	0^oC
T_4	4^oC
T_5	30^oC(room temperature)

The betalain content was measured on alternate days for first 7 days and at weekly intervals up to 28 days. The betalain content was measured using citrate phosphate buffer and expressed as mg/l.

2.8 Stability of betalain extract of Gompherna at different light intensity

Concentrated betalain extracts were taken in screw-capped vials and stored under ambient conditions at different light intensity as follows,

Treatments	**Light levels**
T_1	Dark
T_2	565 lux
T_3	1140 lux

The above light intensity ranges were fixed based on earlier reports. White incandescent light was used as light source. The betalain content was measured on alternate days for first 7 days and at weekly intervals up to 28 days. The betalain content was measured using citrate phosphate buffer and expressed as mg/l.

2.9 Sensory Scoring of Gompherna tea and Ice cream

5g of dried Gompherna petals were used for the making tea at 100ml of water. Since the pigment is highly soluble in water, they readily dissolved in water by giving a Purple to violet colour. The Gompherna tea is compared to the Normal Black Tea. And for Ice cream, 30mg of the lyophilized extract of Gompherna is added to the 100g plain vanilla Ice cream to impart colour in it and it is compared to the plain non coloured Vanilla Ice cream. Scoring of the products was done by a panel of members consisting of educated professors, assistant professors and students of Floriculture and Landscape architecture department at Tamil Nadu Agricultural University (India) based on the Taste, Texture, Flavor, Appearance, Palatability and overall acceptability over the control using five point hedonic scale (1:Extremely good, 5: bad).

2.10 Statistical analysis - The statistical analysis was carried out using IBM SPSS Statistics 20, DSTAT and Graph pad prism 5 software.

3. Results

3.1 Total Betalain Content with respect to Spectroscopy method and HPLC method

The Gompherna showed a higher total betalain content, betacyanin and betaxanthin content of 30.51mg/l, 33.35mg/l and 22.76 mg/l using aqueous solution as a solvent respectively.The HPLC quantification of Gompherna extract for betanin (Betanidin 5-o-glucoside) standard showed several major peaks and minor peaks. The elution was monitored at 535 nm. The major peak detected at the retention time of 8.3 minute was readily identified as betanin (Betanidin 5-o-glucoside). Elution of the flower extract coincided with that of the standard. Based on the Rt and elution of standard and peak, the betanin content was quantified 88.41ppm.

3.2 Anti -oxidant activity of *Gompherna globosa*

3.2.1 Free radical scavenging activity

Significant results were obtained with regard to antioxidant activity of betalain extract. It was revealed that the flower extract scavenged the DPPH and ABTS free radicals depending upon their dosage level.

Antioxidant potential is observed in Gomphrena flower extracts with an IC_{50} value of 181.24mg/ml when compared to ascorbic acid standard (54.23 mg/ml) by DPPH method. The standard ascorbic acid showed IC_{50} at 81.26µg/ml concentration whereas the $IC_{50}$of Gompherna flower extract is 27.2mg/ml by ABTS method.

3.2.2 Total reducing power assay

Significant results were obtained with regard to antioxidant activity of betalain extract by Total reducing power assay. By Chelating potential, the chelates get reduced systematically at higher concentration of betalain extract and results found that Gomphrena has 50% inhibition at 2.229mg/ml whereas, the IC_{50} value of ascorbic acid standard is 7.17µg/ml respectively.

By CUPRAC (Cupric reducing antioxidant power) method and FRAP (Ferric ion reducing antioxidant power) method,irrespective of IC_{50} value, the antioxidant potential is given in terms of µg equivalence to standard (*i.e.*) ascorbic acid by CUPRAC method. Gomphrena showed the anti-oxidant potential as 49.05µg equivalence to that of ascorbic acid by CUPRAC. FRAP method exhibited 106.17µg equivalence to that of standard in Gompherna flower extract.

3.3 Anti -Microbial activity

The data on antimicrobial activity of *Gomphrena globosa* against three bacteria *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and two fungi (*Rhizopus spp*and *Aspergilles niger*) recorded significant results. Among the bacterial cultures highest inhibition zone of 2.98cm RIZD was observed against *Bacillus subtilis*. With regard to fungal cultures higher antimicrobial potential was registered against *Aspergilles niger (*3.52cm RIZD) at 500mg/ml concentration. Lowest inhibition zone was observed against *Rhizopus spp*with 1.78 cm of RIZD. No inhibition was observed at the concentration of betalain extracts from 100-300mg/ml against*Escherichia coli*.

3.4 Stability to different light and Temperature

Betalain extract of *Gomphrena globosa* exhibited significant results with regard to storage stability at different light intensities after a week of storage period.

Among the different light intensities, betalain extract stored under dark (T_3) condition showed the highest stability on 1st, 3rd, 5th and 7th day (25.92, 25.31, 24.89mg/l of betalain content respectively) followed by pigments stored at 565lux (T_2) intensity (25.3, 24.98, 24.01 mg/ml of betalain content respectively). Least stability of betalain extract was observed in case of pigments stored at1140lux (T_1) light intensity as the degradation was faster (25.15, 24.01, 22.90mg/l of betalain content).

The stability of betalain extracts from *Gomphrena globosa* was significantly influenced by different storage temperatures. The lowest stability was observed in case of pigments stored at 30°C (17.7, 17.1, and 16.2, 15.02 mg/l of betalain content on 1st, 3rd, 5th and 7th day of storage. Higher stability was observed when pigments were stored at -80°C (21.89, 21.7, 21.57, 21.37 and 21.32mg/l of betalain content) on 5th, 7th, 14th, 21st and 28th day of storage followed by storage at -20°C (21.62, 21.58, 21.32, 21.28, 20.92mg/l of betalain content) after 5th, 7th, 14th, 21st and 28th day of storage. Degradation was less when pigments were stored at 8°C even up to 28 days.

3.5 Sensory evaluation of Gompherna Tea and Ice cream

The Results was confirmed based on the acceptance of 80% respondent of the overall respondent. On comparing the Gompherna tea and Black tea, the acceptability for the colour, Texture and taste was higher to Gompherna tea. The overall acceptability was higher to Gompherna tea due to its taste.

On comparing the plain Vanilla Ice-cream with beautifully coloured Gompherna flavoured Ice-cream was preferred more due to its pleasing and colourful appearance, taste, texture and Palatability by 80% respondent.

DISCUSSION

Gomphrena is reported to have Gomphrenin-I (Minale *et al.*, 1966).Betanin is a sub group of betacyanins which are further classified as amaranthin, gomphrenin and decarboxy-betanin group (Strack *et al.*, 1980). So on comparing the Spectroscopy and HPLC method Gompherna extract holds more Betacyanin content.

The betacyanin and betaxanthin content were reported for the scavenging activity thereby inhibiting oxidation. Various methods are reported for the analysis of antioxidant potential of Gompherna pigments. The antioxidant potential of a compound is confirmed by analyzing the free radical scavenging activity and total reducing power using ABTS, DPPH, Chelating potential, FRAP, PFRAP and CUPRAC methods. Betalains were reported to have higher antioxidant property as illustrated (Escribano *et al.*, 1998). Betalains contain a cyclic amine which is similar in chemical structure of the antioxidant ethoxyquine(Luisa Tesoriere *et al.*, 2004).

The Gompherna extracts showed a higher anti-microbial activity against different food pathogens. The inhibition zone formed against *Pseudomonas aeruginosa* in the present study corroborates with the work of previous workers Hamiduzzaman *et al.*, (2012)in *Gomphrena globosa* which inhibited to a diameter of 14mm. Therefore, betalain extract from *Gomphrena globosa*is a potential food dye with good properties.

Betalain pigment from Gompherna when stored at different temperature and light conditions they show a greater degradation at room temperature and high light intensity. Hence betalains very highly sensitive to light and temperature and are required to be stored under refrigeration and in dark. This was in accordance with Reshm *et al.*(2012)which was reported in betalain extracts of *Basella alba* fruit and (Castellar *et al.*, 2003) in *Opuntiastricta.*This difference in stability might be due to the breakage caused by light in the double bond of the electron in the betacyanin molecule which is in the excited stage resulting in the destruction of the betacyaninandthat pigment degradation was influenced by many factors like pH, light and heat and not only by the temperature.

CONCLUSION

Gomphernaglobosa a potential plant for betacyanin and to be used as food dye for their health benefits and aesthetic property that creates a appealing visual especially in the frozen products like Yogurt, Candies, Ice-cream, Squash etc.,Further studies can be forwarded in terms of different extraction method and analyzing other different potential benefits.

REFERENCES

Brand-Williams W, Cuvelier M.-E, & Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology *28*(1): 25-30.

Castellar, Obon, & Fernandez-Lopez (2006) The isolation and properties of a concentrated red-purple betacyanin food colourant from Opuntia stricta fruits. Journal of the Science of Food and Agriculture 122-128. doi: 10.1002/jsfa.2285

Castellar R N, J. M. O, Alacid M, & Pez J (2003) Color Properties and Stability of Betacyanins from Opuntia Fruits. J. Agric. Food Chem51: 2772−2776.

Custódio Lobo Roriz, João C.M. Barreira, Patricia Morales, Lillian Barros, Isabel C.F.R. Ferreira (2019) Gomphrena globosa L. as a novel source of food-grade betacyanins: Incorporation in ice-cream and comparison with beet-root extracts and commercial betalains. LWT - Food Science and TechnologyS0023-6438(18)30138-5.

Fernández-López J, Castellar R, Obón J, & Almela L (2002) Screening and mass-spectral confirmation of betalains in cactus pears. Chromatographia 56(9-10):591-595.

Hamiduzzaman M, & Azam A T M Z (2012) Antimicrobial, Antioxidant and Cytotoxic Activities of Gomphrena globosa (L.). Bangladesh Pharmaceutical Journal15(2):183-185.

Karawita R, Siriwardhana N, Lee, K.-W, Heo, M.-S, Yeo, I.-K, Lee, Y.-D, & Jeon, Y-J (2005) Reactive oxygen species scavenging, metal chelation, reducing power and lipid peroxidation inhibition properties of different solvent fractions from Hizikia fusiformis. European Food Research and Technology *220*(3-4): 363-371.

Kumar R, Mishra A K, Dubey N, & Tripathi Y (2007) Evaluation of Chenopodium ambrosioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. International journal of food microbiology115(2): 159-164.

Minale, L., Piattelli, M., De Stefano, S., & Nicolaus, R. (1966). Pigments of centrospermae—VI.: Acylated betacyanins. *Phytochemistry*, *5*(6), 1037-1052.

Moßhammer M R, Stintzing F C, & Carle (2005) Colour studies on fruit juice blends from Opuntia and Hylocereus cacti and betalain-containing models olutions derived therefrom. Food Research International 38:975–981. doi: 10.1016/j.foodres.2005.01.015

Reshm S K, Aravindhan K M, & Devi P S (2012) The effect of light, temperature, pH on stability of betacyanin pigments in *Basella alba* fruit. Asian Journal of Pharmaceutical and Clinical Research5(4):107-110.

Sabarudin N A, Munaim M S A, & Wahid Z A (2016) Effect of extraction condition of natural dye pigment from Bougainvillea flowers' bract. Australian Journal of Basic and Applied Sciences *10*(17):172-175.

Sahreen S, Khan M R, & Khan R A (2010) Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food chemistry122(4): 1205-1211.

Strack D, Engel U, & Reznik H (1980) High Performance Liquid Chromatography of Betalains and its Application to Pigment Analysis in Aizoaceae and Cactaceae. z. PJlanzenphysiol. Bd 101: 215-222. doi: 10.1016/S0044-328X(81)80027-X

Wong S P, Leong L P, & Koh J H W (2006) Antioxidant activities of aqueous extracts of selected plants. Food chemistry 99(4): 775-783.

Yıldız L, Başkan K S, Tütem E, & Apak R (2008) Combined HPLC-CUPRAC (cupric ion reducing antioxidant capacity) assay of parsley, celery leaves, and nettle. Talanta 77(1): 304-313.

Antioxidant potential of *Gompherna globosa* by ABTS, DPPH, and chelating potential method

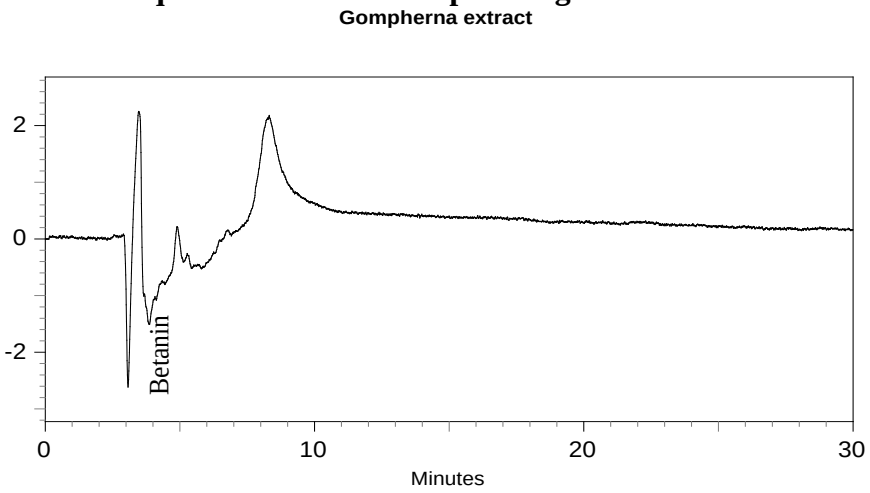
Crop	ABTS(IC_{50})	DPPH(IC_{50})	Chelating potential(IC_{50})
Gomphrena globosa (mg/ml)	27.2 ± 0.24^{b}	181.243 ± 3.26^{c}	2.229 ± 0.09^{c}
CD value	0.36	2.37	0.08
SE(d)	0.18	1.18	0.04
Ascorbic acid (std) (µg/ml)	81.26 ± 0.43	54.23 ± 0.22	7.17 ± 0.07

Anti-microbial potential of *Gompherna globosa* on different microorganism

Concentration of *Gomphrena globosa*extract	**Diameter of inhibition zone (cm)**				
	Microorganisms				
	Bacillus subtilis	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergilles niger*	*Rhizopus spp*
100 mg/ml	2.2 ± 0.01^{c}	0.79 ± 0.01^{c}	0^{c}	0.56 ± 0.01^{d}	1.26 ± 0.03^{d}
200 mg/ml	2.63 ± 0.07^{b}	2.45 ± 0.007^{b}	0^{c}	2.18 ± 0.02^{c}	1.59 ± 0.02^{c}
300 mg/ml	2.68 ± 0.006^{b}	2.70 ± 0.015^{a}	0^{c}	2.58 ± 0.05^{b}	1.69 ± 0.04^{bc}
400 mg/ml	2.73 ± 0.02^{b}	2.76 ± 0.06^{a}	0.79 ± 0.004^{b}	3.33 ± 0.01^{a}	1.78 ± 0.05^{b}
500 mg/ml	2.98 ± 0.06^{a}	2.82 ± 0.04^{a}	1.49 ± 0.03^{a}	3.52 ± 0.05^{a}	2.32 ± 0.06^{a}
Positive control(mm) 1µg/ml	16.5 ± 2.5	18.5 ± 2.5	12.5 ± 0.5	17.2 ± 1.5	11.0 ± 0.5
CD Value	0.08	0.08	0.04	0.11	0.07
SE(d)	0.04	0.04	0.02	0.05	0.03

The values are represented as mean ± SD with triplicate determination

HPLC quantification of Gompherna globosa for Betanin



Effect of Light on stability of betalain pigment extracted from *Gomphrena globosa*

Light intensity (LUX)	**Betalain content(mg/ml)**					
	Storage period in days					
	0	**1**	**3**	**5**	**7**	**14**
T_1(1140)	26.53± 0.25^{a}	25.15 ± 0.11^{a}	24.01 ± 0.28^{ab}	22.90 ± 0.88^{ab}	0^{b}	0^{c}
T_2(564)	26.5375 ± 0.25^{a}	25.34 ± 0.70^{a}	24.98 ± 0.02^{a}	24.01± 1.03^{a}	0^{b}	0^{c}
T_3(Dark)	26.53± 0.25^{a}	25.92± 0.25^{a}	25.31 ± 0.79^{a}	24.89±0.53^{a}	0^{b}	0^{b}
	Light(L)	**Days(D)**	**LxD**			
SE(d)	0.18	0.25	0.44			
CD	0.36	0.51	0.89			

The values are represented as mean ± SD with triplicate determination

Effect of storage temperature on stability of betalain pigment extracted from *Gomphrena globosa*

Storage temperature (°C)	**Betalain content(mg/ml)**							
	Storage period in days							
	0	1	3	5	7	14	21	28
-80	21.68± 0.63^{a}	21.67± 0.03^{a}	21.62± 0.64^{a}	21.52± 0.50^{a}	21.48± 0.07^{a}	21.3± 0.74^{a}	21.27± 0.23^{a}	20.98± 0.61^{a}
-20	21.68± 0.63^{a}	21.65± 0.46^{a}	21.58± 0.33^{a}	21.51± 0.21^{a}	21.41± 0.67^{a}	21.29± 0.72^{a}	20.89± 0.01^{a}	20.78± 0.55^{a}
0	21.68± 0.63^{a}	21.57± 4.35^{a}	21.42± 0.81^{a}	21.38± 0.28^{a}	21.19± 0.22^{a}	20.91± 0.92^{a}	20.71± 0.07^{a}	20.45± 0.90^{a}
4	21.68± 0.63^{a}	20.95± 0.37^{a}	20.65± 0.07^{a}	20.48± 0.59^{a}	20.27± 0.71^{a}	20.13± 0.78^{a}	20.03± 0.74^{a}	19.82± 0.32^{a}
8	21.68± 0.63^{a}	19.89± 0.860^{a}	19.82±0. 10^{a}	19.75± 0.24^{a}	19.63± 0.23^{a}	19.38± 0.85^{a}	19.27± 0.15^{a}	18.89± 0.50^{a}
30	21.68± 0.63^{a}	18.69± 0.47^{a}	18.21± 0.36^{a}	17.95± 0.17^{a}	0^{b}	0^{b}	0^{b}	0^{b}
	Temperature(T)	Days(D)	TxD					
SE(d)	0.15	0.17	0.43					
CD	0.30	0.35	0.87					

The values are represented as mean ± SD with triplicate determination