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Exploring the potentiality of natural food-grade betacyanin from *Gomphrena globosa*

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Abstract
The synthetic dye used in the food, textiles and pharmaceutical industry holds a negative impact on the health and the environment causing ill diseases to humans and pollution to the environment. Considering the significance of natural colourants in the food industry, the present study aimed to bring the benefits and potentiality of Betalain, a bio-pigment into the limelight as a food dye extracted from *Gomphrena globosa*. The pigment has been extracted using the aqueous solution as a solvent and they are quantified by Spectroscopic method and HPLC method and the extracts were characterized for Anti-oxidant, Anti-microbial property. The storage of Gompherna extract at different temperatures and light intensities were also measured. 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 their appealing colour, Texture, Taste and Palatability.

Keywords: *Gomphrena globosa*, quantification, anti-oxidant, anti-microbial, stability and food dye

Introduction
Synthetic dyes and pigments are the major pollutants affecting the environment, soil and water resources and thereby cause health issues to humans. To overcome the harmfulness caused by synthetic dyes to humans and the environment, researchers have been involved in the development of natural dyes and pigment (Kumar *et al.*, 2017) [7]. Betalains were one of the natural pigments found in Plants which is a potential food dye giving a wide range of colours from red to Violet. Apart from the use as a colourant, betalains have a wide range of biological activities with potential health benefits like they counter inflammation, protecting the liver and having anticancer, antitumor and antioxidant properties. Gomphrena species is an edible, Ornamental and medicinal plant commonly known as Globe Amaranth or Bachelor Button, which belongs to the family Amaranthaceae. Though extraction procedures for betalains have been reported in crops like red beet, and *Opuntia*, research on the extraction of betalain pigments from flowers is meagre and needs to be exploited. Considering the importance of flowers as a source of natural betalain pigments, the present paper aimed to extract, quantify and expose the antioxidant, antimicrobial properties and stability of betalains from *Gomphrena globosa* and their potential usage as a food dye.

Materials and Methods
Sample preparation
One gram of shade-dried Gompherna petals was 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.

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 600 nm for the quantification of betacyanin, betaxanthin, and total betalains respectively using UV-VIS spectrophotometer (Eppendorf bio spectrometer) (Moßhammer *et al.*, 2005) [9].
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^{-1} 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^{-1}; and molar extinction coefficients (ε) = 48 000 L/ (mol cm) in H_2O; λ=480 nm

Quantification by HPLC
Betanin was quantified by a water modular liquid chromatographic system (Shimadzu LC-88 A) equipped with two M510 pumps, an M996 photodiode array detector and a rheodyne model 7125 injector and a sample loop of 20 µl was used, along with a Millenium 2010 chromatography data management system. A kromasil 100 $C_{18,}$ 5 µM, 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) [4]. 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^{-1}. The betanin standard and Gompherna extract were diluted with HPLC grade distilled water and the concentration is maintained at 100 ppm.

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 Total Reducing power assay. For the estimation of antioxidants, the Gompherna extract was prepared at different concentrations using distilled water *viz*., 1250, 1000, 750, 500, 250 µg ml^{-1}.

Free radical scavenging activity: The betalain extract was measured for antioxidant activity by its ability to scavenge the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS by (Wong *et al.*, 2006) [14] with slight modifications.
Ascorbic acid at different concentrations (1 mg ml^{-1} to 5 mg ml^{-1}) was used as a standard for both 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) [15].

Total reducing power assay: FRAP (Ferric ion reducing antioxidant power) method and CUPRAC (Cupric reducing antioxidant power) method was followed by (Brand-Williams *et al.*, 1995) [1] and (Sahreen *et al.*, 2010) [12] with some slight modifications and it is expressed as µM Ascorbic acid equivalent. The chelating potential was analyzed by adding the sample with $FeCl_2$. $2H_2O$ (2.0 mM) and ferrozine The chelating activity (%) was calculated using the above equation for per cent inhibition.

Assessment of antimicrobial properties of betalain extracts
The Gompherna extracts were analysed for the antimicrobial activity by Agar well diffusion method (Castellar *et al*, 2006) [12] against the following foodborne bacteria and fungi with the given concentrations 100 mg ml^{-1}, 200 mg ml^{-1}, 300 mg ml^{-1}, 400 mg ml^{-1} and 500 mg ml^{-1}. The diameter of the inhibition zone (Yıldız *et al.*) [15] was measured and the mean D1Z was calculated. The antimicrobial activity was assessed by calculating the relative inhibition zone diameter (RIZD).

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

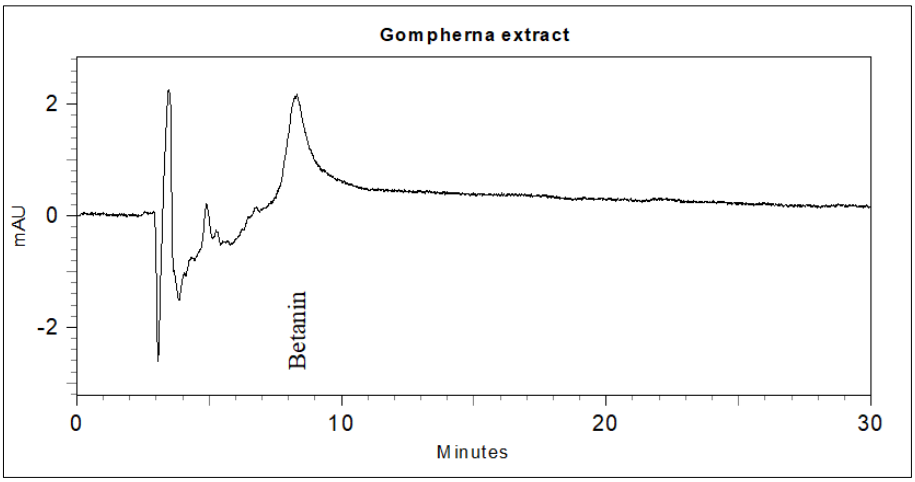
Microorganisms tested: *Escherichia coli* (O157 strain), *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Rhizopus* spp, *Aspergillus niger*.

Stability of betalain extract of Gompherna at different temperatures and light intensity
The effect of different storage temperatures and Light intensity on betalain stability was analyzed. The concentrated extracts were taken in screw-capped vials and stored at different temperatures (-80 °C, -20 °C, 0 °C, 4 °C, 30 °C) and different light intensity (Dark, 565 lux, 1140 lux). The betalain content was measured on alternate days for the 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.

Sensory Scoring of Gompherna tea and Ice cream
5 g of dried Gompherna petals were used for making tea at 100 ml of water. Since the pigment is highly soluble in water, it 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, 30 mg of the lyophilized extract of Gompherna is added to the 100 g plain vanilla Ice cream to impart colour to 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 the 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 a five-point hedonic scale (1:Extremely good, 5: bad).

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

Results and Discussion
Total Betalain Content concerning 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 the 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 (Graph 1). The elution was monitored at 535 nm. The major peak detected at the retention time of 8.3 minutes was readily identified as betanin (Betanidin 5-o-glucoside). The 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 at 88.41ppm.



Graph 1: HPLC quantification of *Gomphrena globosa* for Betanin

Anti-oxidant activity of *Gomphrena globosa*

Free radical scavenging activity: Significant results were obtained about the antioxidant activity of betalain extract. It was revealed that the antioxidant potential by the DPPH, ABTS and Chelating potential depends on their dosage level. The antioxidant potential is observed in Gomphrena flower extracts with an IC_{50} value of 181.24 mg ml^{-1} when compared to ascorbic acid standard (54.23 mg ml^{-1}) by the DPPH method. The standard ascorbic acid showed IC_{50} at 81.26 µg/ml concentration whereas the IC_{50} of Gompherna flower extract is 27.2 mg ml^{-1} by ABTS method. By Chelating potential, the Gomphrena has 50% inhibition at 2.229 mg ml^{-1} whereas, the IC_{50} value of the ascorbic acid standard is 7.17 µg/ml respectively (Table 1).

Total reducing power assay: 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 49.05 µg equivalence to that of ascorbic acid by CUPRAC. FRAP method exhibited 106.17 µg equivalence to that of standard in Gompherna flower extract (Table 1).

Table 1: Antioxidant potential of *Gomphrena globosa* by ABTS, DPPH, and chelating potential method

Crop	ABTS(IC_{50})	DPPH(IC_{50})	Chelating potential (IC_{50})
Gomphrena globosa (mg ml^{-1})	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 activity: The data on antimicrobial activity of *Gomphrena globosa* against three bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and two fungi (*Rhizopus spp* and *Aspergillus niger*) recorded significant results. Among the bacterial cultures highest inhibition zone of 2.98cm, RIZD was observed against *Bacillus subtilis*. Concerning fungal cultures, the higher antimicrobial potential was registered against *Aspergillus niger* (3.52cm RIZD) at 500mg ml^{-1} concentration. The 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^{-1} against *Escherichia coli* (Table 2).

Table 2: The anti-microbial potential of *Gomphrena globosa* on different microorganisms

The concentration of *Gomphrena globosa* extract	Diameter of inhibition zone (cm)				
	Microorganisms				
	Bacillus subtilis	***Pseudomonas aeruginosa***	***Escherichia coli***	***Aspergillus niger***	***Rhizopus spp***
100 mg ml^{-1}	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^{-1}	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^{-1}	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^{-1}	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^{-1}	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

Stability to different light and Temperature: Betalain extract of *Gomphrena globosa* exhibited significant results concerning storage stability at different light intensities after a week of storage period. Among the different light intensities, betalain extract stored under dark (T_3) conditions showed the highest stability on 1st, 3rd, 5th and 7th day (25.92, 25.31,

24.89 mg/l of betalain content respectively) followed by pigments stored at 565 lux (T_2) intensity (25.3, 24.98, 24.01 mg ml^{-1} of betalain content respectively). The least stability of betalain extract was observed in the case of pigments stored at 1140 lux (T_1) light intensity as the degradation was faster (25.15, 24.01, 22.90 mg/l of betalain content) (Table 3).

The stability of betalain extracts from *Gomphrena globosa* was significantly influenced by different storage temperatures. The lowest stability was observed in the case of pigments stored at 30 °C (17.7, 17.1, and 16.2, 15.02 mg/l of betalain content on the 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, 20.92 mg/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 (Table 4).

Table 3: Effect of Light on the stability of betalain pigment extracted from *Gomphrena globosa*

Light intensity (LUX)	Betalain content(mg ml^{-1})					
	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

Table 4: Effect of storage temperature on the stability of betalain pigment extracted from *Gomphrena globose*

Storage temperature (°C)	Betalain content(mg ml^{-1})							
	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)							
SE(d)	0.15							
CD	0.30							

The values are represented as mean±SD with triplicate determination

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% of respondents.

Discussion

Gomphrena is reported to have Gomphrenin-I (Minale *et al.*, 1966) [8]. Betanin is a sub group of betacyanins which are further classified as amaranthin, gomphrenin and decarboxy-betanin group (Strack *et al.*, 1980) [13]. 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. Betalains contain a cyclic amine which is similar in chemical structure of the antioxidant ethoxyquine.

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) [5] 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) [10] which was reported in betalain extracts of *Basella alba* fruit and (Castellar *et al.*, 2003) [12] in *Opuntia stricta.* 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 betacyanin and that pigment degradation was influenced by many factors like pH, light and heat and not only by the temperature.

Conclusion: *Gomphrena globosa* is a potential plant for betacyanin and to be used as a food dye for their health

benefits and aesthetic property that creates an appealing visual, especially in 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.

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