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BOUGAINVILLEA BRILLIANCE: DES-DRIVEN GREEN EXTRACTION, QUANTIFICATION, AND NANOENCAPSULATION OF BETALAINS FOR NATURAL COLOURING

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ABSTRACT

Synthetic food colourants pose environmental and health risks, necessitating sustainable natural alternatives. This study optimized green extraction, quantification, and encapsulation of betalains from *Bougainvillea spectabilis* flowers using deep eutectic solvents (DES) at the Department of Floriculture, TNAU, employing a completely randomized design (CRD) with seven treatments. Choline chloride–citric acid DES (T_6) achieved the highest yields: 66.24 mg g^{-1} betacyanins, 56.86 mg g^{-1} betaxanthins, 123.10 mg g^{-1} total betalains, and 24.18 mg g^{-1} total phenols. Betalain stability was optimal at “20°C (106.94 mg g^{-1}) versus 30°C. HPLC identified betanin (98.30 ppm, retention time 8.30 min). Spray-dried powder exhibited 68% recovery, 98.32% solubility, 16.84% hygroscopicity, 0.32% fibre, and desirable colour (L* = 38.10, a* = +29.40, b* = +2.30). Sensory evaluation of model foods (cake cream, pudding, RTS beverages) showed high acceptability. Choline chloride–citric acid DES enables efficient, scalable production of bioactive *B. spectabilis* betalains as natural food colourants.

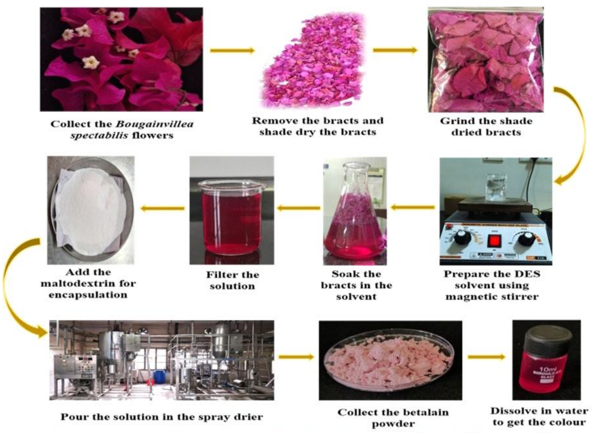
***Key words*:** Bougainvillea; betalains; deep eutectic solvents; spray drying; encapsulation; natural colourant.

Introduction

Flowers represent a versatile source of natural colourants, offering both aesthetic value and functional pigments for applications in food, textiles, cosmetics, and pharmaceuticals. Growing concern over the carcinogenicity and eco-toxicological impact of synthetic dyes and food colours has renewed interest in bio-based colourants, despite practical challenges such as limited stability and comparatively higher production costs (Bashir *et al.*, 2024). Among natural pigments, betalains are water-soluble nitrogenous compounds occurring in species such as Opuntia, Bougainvillea, Gomphrena, and Celosia are especially attractive due to their intense colour and favourable safety profile. Betanin (E162), the principal commercial betalain, is widely employed as a natural red colourant in foods and related products, and is further distinguished by its reported antioxidant, anti-inflammatory, hepatoprotective, and potential chemo preventive activities (Ardila-Leal *et al.*, 2021). These bioactivities, together with high bioavailability and retention of antioxidant capacity in the gastrointestinal tract, support the positioning of betalain-rich extracts as functional ingredients with added health value (Patil *et al.*, 2025).

Parallel advances in green chemistry have identified Deep Eutectic Solvents (DES) as promising alternatives to conventional organic solvents for the extraction and stabilization of bioactive compounds. DES, typically composed of biodegradable, low-toxicity, and often food-grade components, can be tailored to dissolve both hydrophilic and hydrophobic molecules, thereby improving extraction efficiency while preserving pigment integrity and bioactivity (Hansen *et al.*, 2021). Applying DES to the extraction of betalains from ornamental flowers such as *Bougainvillea spectabilis* provides a sustainable route to high-value natural colourants that can replace synthetic food colours associated with human health and environmental risks. The present study therefore investigates flower-derived betalains as DES-extracted colourants, with a focus on their safety, stability, and functionality, aiming to support their broader deployment as eco-friendly, health-promoting alternatives in food systems.

Graphical abstract



Green extraction and encapsulation of betalain pigment from *Bougainvillea spectabilis*

Materials and methods

Study area and extraction of betalains

The study was conducted at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore (11°072 N, 77°592 E; 426.26 m MSL), utilizing *Bougainvillea spectabilis* flower bracts in a Completely Randomized Design (CRD) with seven treatments and three replications. Shade-dried flowers were powdered, and DESs were prepared by heating choline chloride (HBA) with hydrogen bond donors (HBDs) at 50–100°C until homogeneous. Extractions involved 1 g powder in 10 mL DES at 50°C for 30–60 min, followed by centrifugation (10,000 rpm, 10 min). Extracts were analyzed for betacyanin (538 nm UV-Vis), betaxanthin, total betalains, and total phenolic content (Folin–Ciocalteu assay, 765 nm).

Treatment combination

T_1- Control (Distilled water)
T_2- Choline chloride + Ascorbic acid
T_3- Choline chloride + Ethylene glycol
T_4- Choline chloride + Glycerol
T_5- Choline chloride + Glucose
T_6- Choline chloride + Citric acid
T_7- Choline chloride + Malic acid

Stability and Quantification of betalains

Betalain stability was assessed for optimal DES of Bougainvillea betalain extract. 1 g powder soaked in 10 mL DES at 50°C for 45 min, centrifuged, and stored at -20°C, 0°C, 4°C, or 30°C for 30 days in transparent vials, with retention calculated as (A_t/A_0) ×100 via 538 nm absorbance every 5 days. Betanin was quantified by HPLC (Shimadzu LC-88 A: M510 pumps, M996 PDA detector, Rheodyne 7125 injector/20 µL loop; Millenium 2010 software) on Kromasil C18 (5 µm, 25 cm × 4.6 mm) with 1% acetic acid in water/acetonitrile at 1 mL min and100 ppm standard in HPLC water, extracts reconstituted in 80% methanol, peaks identified by retention time and UV-Vis spectra.

Physiochemical analysis of spray dried powder of extract

A pilot-scale spray dryer (25 L, GOMA Engineering Pvt. Ltd., Mumbai) at the Center for Post-Harvest Technology, TNAU converted extracts to powder (180/80°C inlet/outlet (Shofinita *et al*., 2023). Physicochemical analyses included: total sugar (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956), crude fiber (Ranganna, 1986), recovery of powder (Goula & Adamopoulos, 2005), solubility (Cano Chauca *et al*., 2005), hygroscopicity (Ratti, 2001), and colour (L*, a*, b* via 3nh spectrophotometer) RHS chart (Strack, *et al*., 2003).

Value addition and sensory evaluation of betalain powder as natural food colour

Powder was incorporated as a natural colourant per *fssai* guidelines into cake cream (2.5 g/100 g), RTS (3.0 g/250 mL), lemon juice (2.0 g/250 mL), and pudding (2.5 g/100 g), evaluated sensorially on a 5-point hedonic scale (Ebuehi *et al*., 2007).

Hedonic scale:

Score range	Acceptance level
1.50 – 2.49	Not acceptable
2.50– 3.49	Slightly acceptable
3.50 – 4.49	Moderately acceptable
4.50 – 5.00	Highly acceptable

Data underwent angular transformation, CD at 5% significance via AGRIS software and Excel.

Results and Discussion

Ideal DES solvent for betalain extraction

Bougainvillea spectabilis flower extracts treated with choline chloride + citric acid (T_6) exhibited highest betalain levels (66.24 mg g^{-1} betacyanin, 56.86 mg g^{-1} betaxanthin, and 123.10 mg g^{-1} total betalains) as shown

Table 1: Estimated betalain composition (Betacyanin, Betaxanthin & Total betalain) and phenol content in *Bougainvillea spectabilis* flower extract (mg g^{-1})

Treatment	Betacyanin (mg g^{-1})	Betaxanthin (mg g^{-1})	Total betalain (mg g^{-1})	Phenol content (mg g^{-1})
T_1: Distilled Water	28.76	23.38	52.14	13.39
T_2: Choline Chloride + Ascorbic acid	52.39	46.18	98.56	21.20
T_3: Choline Chloride + Ethylene glycol	55.79	47.70	103.49	24.18
T_4: Choline Chloride + Glycerol	42.38	35.44	77.82	22.42
T_5: Choline Chloride + Glucose	34.81	27.53	62.34	17.39
T_6: Choline Chloride + Citric acid	66.24	56.86	123.10	12.09
T_7: Choline Chloride + Malic acid	52.75	44.46	97.21	21.46
Mean	47.59	40.22	87.81	19.00
SE(d)	1.66	1.56	2.10	0.82
CD (0.05)	3.56	3.34	4.51	1.75

in Table 1, eclipsing yields from other DES variants. Citric acid excels here by acting as both hydrogen bond donor and pH buffer, safeguarding betalains' immonium chromophores in acidic milieu to maintain integrity, elevate solubility, and promote vacuolar pigment liberation (Demuner *et al.*, 2023; Rosa *et al.*, 2023). By contrast, glucose-DES (T_5) lagged owing to its high viscosity, which curbs diffusion and cell wall ingress, impeding pigment mass transfer. Such outcomes reinforce choline chloride DES as premier sustainable options over hazardous organics, delivering enhanced yields and environmental benefits for betalain recovery (Gul *et al.*, 2015).

In *Bougainvillea spectabilis* betalain extracts, choline chloride + ethylene glycol (T_3) yielded the highest total phenol content at 24.18 mg g^{-1} (Table 1), versus the lowest 12.09 mg g^{-1} from choline chloride + citric acid (T_6) and 13.39 mg g^{-1} in control (T_1). Solvent type governs phenolic extraction from betalain matrices; low-phenol extracts like those from T_6 suit food applications emphasizing colour. DES stand out for polyphenol recovery via hydrogen-bonded eutectics and acidic shielding against oxidation (Idham *et al.*, 2022; Kumar *et al.*, 2023).

Table 2: Stability of betalain composition (mg g^{-1}) in *Bougainvillea spectabilis* flower extract at various storage temperatures.

Temperature / Storage days	Betalain composition (mg g^{-1})			
	-20°C	-0°C	4°C	30°C
1	123.10	123.10	123.10	123.10
5	122.10	121.78	121.11	119.15
10	120.05	119.42	119.08	116.25
15	117.17	115.69	113.36	103.82
20	114.18	106.97	102.31	93.98
25	110.77	102.53	97.86	76.78
30	106.94	99.20	92.54	59.04
SE(d)	3.57	3.55	2.26	4.16
CD (0.05)	7.65	7.61	4.85	8.92

Optimal temperature for storage

Choline chloride + citric acid (T_6) was the optimal DES for betalain extraction from *Bougainvillea spectabilis*, with Table 2 showing peak stability at -20°C (106.94 mg g^{-1}) and 0°C (99.20 mg g^{-1}) versus sharp decline at 30°C (59.04 mg g^{-1}) due to heat-accelerated hydrolysis, oxidation, and ROS via Arrhenius kinetics (45–

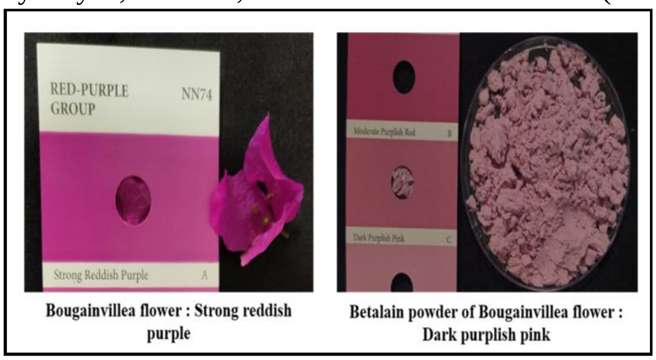
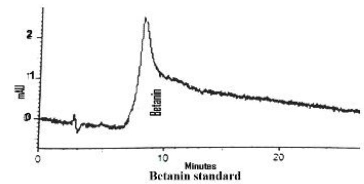
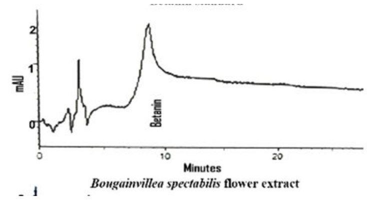


Fig. 1: Colour determination of flower and spray dried betalain powder via RHS colour chart.

Fig. 2: Value added products incorporated with spray dried betalain powder of *Bougainvillea spectabilis.*

Table 3: Quantitative determination of total betalain content by HPLC using betanin as standard.

Standard	λ max (nm)	Retention time (Rt)	Content (ppm)	Chromatogram of HPLC
Betanin (100 ppm)	535	8.331 min	100	
Bougainvillea spectabilis	535	8.35	98.30	

65 kJ mol^{-1}) (Smith *et al.*, 2014). DES hydrogen bonding reduces water activity and mobility, while citric acid's chelation shields chromophores, establishing T6 as a dual extractant-stabilizer for food/nutraceutical shelf life (Prakash *et al.*, 2024; Vijayan *et al.*, 2025; Zhang *et al.*, 2022).

Quantification using HPLC

High-performance liquid chromatography (HPLC) of optimized DES extracts from *Bougainvillea spectabilis* definitively identified betanin (betanidin 5-O-glucoside) as the primary betalain, marked by a key peak at 8.3 min retention time identical to the commercial standard (Table 3), with a concentration of 98.30 ppm that substantiates the extraction and analytical methods' reliability in intricate plant matrices (Borjan *et al.*, 2022). These outcomes emphasize the essential role of precise solvent selection and stringent validation for accurate phytopigment measurement, aligning seamlessly with modern protocols for natural colourant quality assurance and standardization (Nollet & Toldra, 2012).

Table 4: Physiochemical properties of betalain spray dried powder extracted from *Bougainvillea spectabilis* flowers.

Properties		*Bougainvillea spectabilis*
Total sugars (mg g^{-1})		2.74
Crude fibre content (%)		0.32
Powder recovery (%)		68 %
Solubility (%)		98.32
Hygroscopicity (%)		16.84
Colour value (CIELAB)	L*	38.10
	a*	29.40
	b*	2.30
RHS colour chart	Flower	Strong reddish purple (Red purple group NN74)
	Spray dried powder	Dark purplish pink (Greyed purple group 186)

Properties of the betalains spray dried powder extracted from Bougainvillea

The spray-dried betalain powder derived from *Bougainvillea spectabilis* (Table 4) demonstrated advantageous physicochemical characteristics, including total sugars of 2.74 mg g^{-1}, crude fiber at 0.32% reflecting successful purification and encapsulation of soluble components alongside a 68% powder recovery indicative of robust process efficiency. It exhibited exceptional water solubility (98.32%) ideal for beverages and aqueous formulations, moderate hygroscopicity (16.84%) to facilitate handling and prevent caking, and CIELAB colour values of L* 38.10 (moderate lightness), a* +29.40 (pronounced red intensity), and b* +2.3 (negligible yellow shift), RHS chart (Fig. 1) preserving the pigment's vibrant aesthetics post-drying. These traits collectively position the powder as a superior natural colourant with enhanced stability and functionality for food industry applications, in strong agreement with literature on microencapsulated phytopigments (Amchova *et al.*, 2024; Chang *et al.*, 2020; Kaba *et al.*, 2024; Liu *et al.*, 2022; Pieracci *et al.*, 2021; Saini *et al.*, 2024).

Organoleptic evaluation of the betalain powder from Bougainvillea

Spray-dried betalain powder from *Bougainvillea spectabilis* achieved the good sensory acceptability owing

Table 5: Organoleptic characteristics of the processed products coloured with betalain powder from *Bougainvillea spectabilis* flowers.

Products	CO	CL	FV	TT	OA
Butter cream	Mauve	4.6	3.8	4.7	4.7
Lemon juice	Light pink	4.2	3.7	4.4	4.2
RTS	Purple	3.4	3.6	3.9	3.6
Pudding	Rosy pink	4.5	4.4	4.2	4.5
CO: Colour obtained; CL: Colour; FV: Flavour; TT: Taste; OA: Overall Acceptability					

to its superior colour, with organoleptic scores of 4.5 (highly acceptable) for mauve coloured cake cream, 4.4 (acceptable to highly acceptable) for rosy pink coloured pudding, 4.1 for purple coloured RTS, and 3.6 (acceptable) for light pink coloured lemon juice as shown in Table 5, thus validating its efficacy as a natural colourant across matrices (Fig. 2). Elevated ratings in creamy products underscore betalains colour stability and low off-flavor impact (Gomes *et al*., 2024; Kaur *et al*., 2021; Vural & Topuz, 2023), whereas modest scores in acidic/aqueous systems arise from pigment-matrix interactions (Lakshan *et al*., 2020; Madukokila *et al*., 2021; Thanh, Tran, Linh, Vy, & Truc, 2020), encapsulation further safeguards hue and sensory neutrality (Ekie & Evanuarini, 2020), aligning *B. spectabilis* powder as a prime clean-label solution fulfilling visual and functional consumer preferences.

Conclusion

This study establishes *Bougainvillea spectabilis* as a superior source of betalains through optimized deep eutectic solvent (DES) extraction, particularly choline chloride-based combinations with citric acid, yielding stable, high-quality pigments ideal for food colourants. Spray-drying further produced powders with superior solubility, colour retention, and sensory appeal compared to other flower species across various matrices. These results place DES as sustainable alternatives to synthetic solvents, enhancing pigment stability and functionality, with cold storage proven essential for maximizing betalain yields and preserving phytochemical integrity. Overall, DES-extracted betalains from ornamental flowers provide bioactive, clean-label solutions that bridge research and industry demands for natural colourants.

Author Contributions

SS, RC, MG, PG and PA conceived the study design. SS, RC and PG performed data collection and analysis. SS wrote the initial draft. All authors revised and approved the final version.

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Conflicts of Interest: The authors declare no conflicts of interest

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