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Qayoom Sidique
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

NC Shahi
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

UC Lohani
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Anil Kumar
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Om Prakash
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Corresponding Author:
Qayoom Sidique
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Studies on the effect of extraction methods and process parameters on betalain content obtained from *Celosia cristata* L. flowers

Qayoom Sidique, NC Shahi, UC Lohani, Anil Kumar and Om Prakash

Abstract
This study was conducted to understand the different extraction methods *viz* conventional, microwave-assisted extraction, and ultrasound-assisted extraction by extracting the betalain pigments from the waste stalks of *Celosia cristata* and the influence of process parameters *viz*, extraction time, ultrasound power, microwave power, and the ratio of liquid to solid.
Experiments were conducted using the single-factor experiment design wherein one factor was varied at a time keeping other factors constant. Variables selected for the experiment were Temperature (25, 40, 50 °C) & Time (1, 2, 3 h) in case of conventional extraction; Power (150, 175, 200,225, 250 W), Time 10 min, 20 min, 30 min, 40 min, Liquid-Solid ratio (ml/g)10, 20, 30, 40,50 in ultrasound-assisted extraction and Power (160, 320,480,640, 800 W), Time (15, 30, 45, 60, 75 seconds), Liquid-Solid ratio in ml/g (10, 20, 30, 40,50) in microwave-assisted extraction.
The analysis showed the betalain production for betacyanin ranged from 2.98 mg/g dw to 7.02 mg/g dw. The analysis shows that the highest betalain content observed was found to be at 4.46, 5.82, and 7.02mg/g dw in the case of Conventional, Ultrasound-Assisted Extraction, and Microwave-Assisted Extraction respectively.

Keywords: Extraction, betalain, *Celosia cristata* L. flowers

Introduction

The red cockscomb (*Celosia cristata* L.) is a plant from the Amaranthaceae family that is popularly grown as an ornamental plant across the world because of its eye-catching and brightly colored inflorescences. In India, it is mainly found in the Himalayan regions of Jammu and Kashmir, Uttarakhand, and the Northern region of Uttar Pradesh, Himachal Pradesh, and West Bengal. The local people in the Himalayan belt have traditionally used this flower. It is utilized by traditional healers to make different ethnomedicinal compositions and to impart a flaming red colour to gravies without compromising their flavor or scent. (Sayeed, *et al.* 2020) [20]. This is because the plant contains bioactive compounds such astannins, phenolics, flavonoids, kaempferol, sterols, and quercetin, which possess therapeutic activities (Woo *et al.*, 2011) [16].
Betalains are pigments that are water-soluble and found in the cell vacuoles of red beet and flowering plants of the order Caryophyllales. Betalains are classified into betacyanins and betaxanthins based on their structural characteristics and substitution patterns. They have high coloring capacity, are non-toxic and non-allergenic, and offer various health benefits including anti-cancer, anti-inflammatory, anti-lipidemic, antioxidant, and anti-microbial qualities (Schwartz and von Elbe, 1983; Kanner *et al.*, 2001; Cai *et al.*, 2003; Stintzing and Carle, 2007; Clemente and Desai, 2011) [17, 18, 19, 4, 20].
Red beet has been utilized extensively in the food industry as a source of betalain coloring, but its use has been restricted due to microbial contamination, high levels of nitrate, and earthy flavors like Geosmin and 2-sec-butyl-2 methoxypyrazine (Murray and Whitfield, 1975. The *C. cristata* flower, especially the red variety, has the potential to be a promising alternative natural resource to synthetic red color and red beet. This is because it is odorless and does not impart any foul flavor or odor to food. By optimizing the methods and processing variables for the extraction of betalain from novel sources like the *C. cristata* flower, the influence of synthetic colorants on human health can be reduced while meeting the high demand for natural food colors.

The rationale of the present research is to examine methods of extraction and process parameters affecting the extraction of Betalains by the conventional method, non-conventional methods of extraction *viz* ultrasound-assisted extraction and microwave-assisted extraction which can help in the future in using *Celosia cristata* L. as an alternative and promising source of betalain pigments as biocolourant.

Materials and Methods

To achieve the objectives of the study, experimental work was carried out in the following way:

Raw Materials and Chemicals

Flowers of the indigenous red cultivar of *Celosia cristata* L. were obtained from a private nursery in Budgam, Kashmir. For experimental studies, the flower petals were detached from the stalks, and the foliage was removed. The seeds and any remaining dirt were then removed by threshing them. Using the conventional method the *Celosia cristata* blossoms were dried out. To preserve the biologically active elements, the cleaned flowers were shade-dried beneath the roof, omitting the seeds. To create a finely powdered powder, the dried flowers were put through a pulverizing process. The chemicals and reagents employed in the tests were all of the analytical kind. After drying the *C. cristata* flowers were pulverized in a hammer mill to the size of 1-2 mm. The dried powder was stored in glass jars till further processing.

Microwave Assisted/Ultrasound-Assisted Extraction of *Celosia cristata* Flowers

Properly mixed *Celosia cristata* flower powder and solvent mix at different liquid-solid ratios were exposed to microwave radiations of various power and time combinations for the extraction of *Celosia cristata* flower extract. For ultrasound-assisted extraction, properly mixed *Celosia cristata* flower powder and solvent mix at different liquid-solid ratios were subjected to ultra-sonication with different power and time combinations for the extraction of *Celosia cristata* flower extract. Subsequently, the solutions were passed through muslin cloth followed by centrifugation and filtration through filter paper. For further investigation, the supernatant was collected and kept at 4 °C.

Experimental design

In essence, the experimental strategy adopted in this work included a single-factor experimental design, which was utilized for study methods of extraction and process parameters affecting the extraction of Betalains by the conventional method, non-conventional methods of extraction *viz* microwave-assisted extraction, and ultrasound-assisted extraction. Single-factor experiments were conducted by varying one parameter while keeping other parameters constant at the central level. The details are given in Table 1.

Table 1: Experimental Design

S. No	Method	Levels	Response	Reference
01.	Conventional Extraction	Temperature (25, 40, 50 °C) Time (1, 2, 3 h)	Total Betalain Content	Sayeed and Thakur, 2020 [22].
02.	Ultrasound-Assisted Extraction	Power (150, 175, 200, 225, 250 W) Time (minutes)10, 20, 30, 40 Liquid-Solid ratio (ml/g) 10, 20, 30, 40, 50		
03.	Microwave-Assisted Extraction	Power (160, 320, 480, 640, 800 W) Time (15, 30, 45, 60, 75 s) Liquid-Solid ratio (ml/g)10, 20, 30, 40,50		Cardoso-Ugarte, 2014 [23].

Total Betalain Content (TBC)

Total betalain content was determined spectrophotometrically with a UV–VIS spectrometer. The absorbance of extracts was recorded at 536, 538, 430, and 480 nm for quantification of amaranthine, betanin, betalamic acid, and betaxanthin respectively. The Stintzing and Carle (2007) [4] technique was used to determine the total betalain content (TBC).

$$TBC = A_B * DF_A * M_{WA} * \frac{1000}{\varepsilon * L}$$

Where A_B stands for absorption at a certain wavelength; M_{WA} is pigment molecular weight, DF_a is dilution factor; ε is pigment molecular extinction coefficient and L (1 cm) stands for cuvette's route length (1 cm).

The maximum absorbance (λ_{max}) in nm, molecular weight (M_{wA}) in g/mol, and molecular extinction coefficient (ε) in L/mol cm of pigment have been given.

For betalamic acid, λ_{max}= 430,ε= 24,000, M_{wA}= 212.

For betaxanthin, λ_{max}= 480,ε= 48,000, M_{wA}= 309

For amaranthin, λ_{max}= 536,ε= 56,600,M_{wA}= 726.6

For betanin, λ_{max}= 538, M_{wA}= 550, ε= 60,000

Results and Discussions

Effect of extraction method on total betalain content

Table 2 shows the variation of total betalain content among different methods of extraction used. Overall, the results suggest that among all the extraction procedures used, ultrasound-assisted extraction showed the highest total betalain content in CCF extracts. Due to cell wall rupture caused by ultrasonic extraction, which favours betalain diffusion and increases mass transfer, the increased total betalain in ultrasonic-aided extraction may be attributable to these sonochemical effects. (Wen *et al.*, 2020) [1].

Table 2: Highest Total Betalain Content across different methods

Method	Highest Total Betalain Content (mg/g dw)
Conventional Extraction	4.48
Microwave Assisted Extraction	5.81
Ultrasound-Assisted Extraction	6.53

Influence of time and temperature on total betalain content in conventional extraction.

The result in Figure 1 revealed that the maximum amount of pigment content was observed when extraction was carried out at 40 °C as compared to 25 °C. The pigment yield did not increase further upon increasing the temperature from 40°C to 50 °C. The pigment yield first increased with an increase in extraction time from 1 hour to 2 hours but upon a further increase in time above 2 hours, the pigment yield plateaued. A similar trend has been observed by Pandey *et al.*, 2018 [2] while studying the influence of solvent temperature extraction on antioxidant activity, phenolic content, betalain content, and stability of powdered beetroot (*Beta vulgaris* L.) under various storage circumstances.

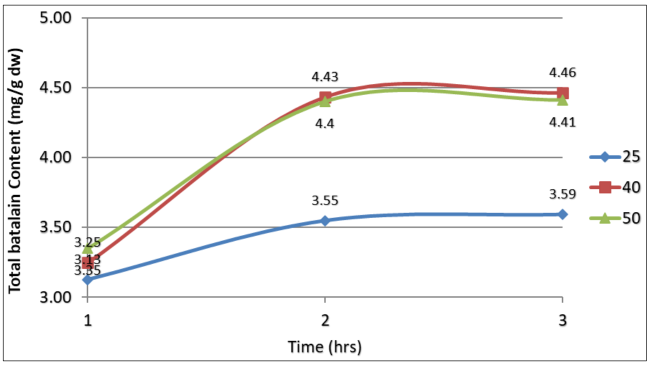

Fig 1: Effect of time and temperature on total betalain content in conventional extraction.

Effect of Microwave extraction time on total betalain content

It is observed from Figure 2 that total betalain content first increased with an increase in microwave time from 15 seconds to 45 seconds where it attained peak and upon a further increase in microwave time total betalain content started decreasing. According to Yansheng *et al.* (2011) [3], microwave treatment increases yield owing to quicker heating rates during microwave treatment in comparison to traditional heating and damage to vegetable tissue from microwave fields. On the other hand, betalains' exposure to high temperatures is to blame for the declining tendency seen at longer extraction durations. Betalains are heat-labile pigments that undergo rapid degradation with increasing temperature and heating time (Herbach, Rohe, Stintzing, & Carle, 2006; Herbach, Stintzing, & Carle, 2004, 2006) [25, 26].

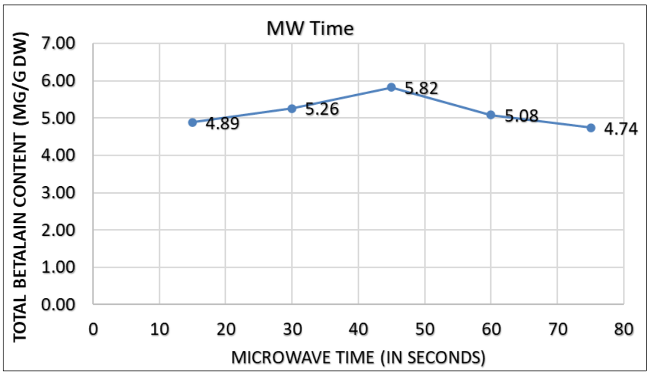

Fig 2: Effect of Microwave time on total betalain content

Effect of Microwave power on total betalain content

It can be observed in Figure 3, that microwave power had a significant effect on the total betalains content. The quantification of betalains increased with an increase in power from 160 W to 480 W and later decreased slightly from 200 to 240 W. Betalains are heat sensitive and inducing the pigment to very high microwave power led to high thermal treatment which in turn changed its colour intensity. Similar cases were reported by Popa *et al.* (2015) [5] in the extraction of betanin from red beet (*Beta vulgaris L.*).

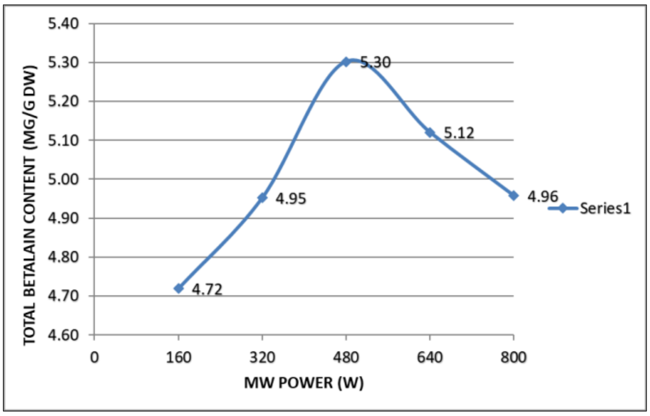

Fig 3: Effect of Microwave Power on Total Betalain Content.

Effect of Solid to Solvent Ratio on Total Betalain Content in microwave Assisted Extraction.

Proper leaching of pigments was greatly influenced by the interface area between solid particles and solvent creating the effect of solid-liquid ratio as one of the major factors (Zhu *et al.*, 2016) [6]. It can be observed from Figure 4 that the liquid-solid ratio had a significant effect on the quantification of betalains. The total betalains content increases with an increase in liquid-solid ratio from 1:10 to 1:30 ml/g and decreases upon further increase from 1:30 to 1:50 ml/g. Similar cases were reported by Popa *et al.*, (2015) [5] in the extraction of betanin from red beet (*Beta vulgaris* L.). According to Al-Farsi and Chang (2007) [7]; Tan *et al.* (2011) [8], a high liquid-solid ratio could promote an increasing concentration gradient, increasing diffusion rate that allows greater extraction of solids by solvent.

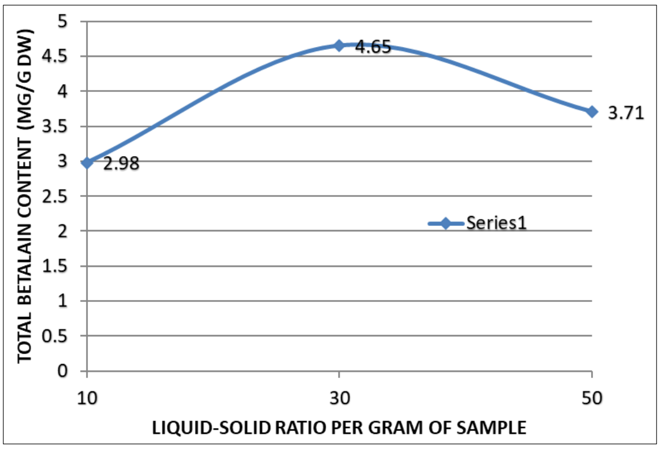

Fig 4: Effect of solid to solvent ratio on total betalain content in microwave assisted extraction

Effect of Ultrasonic Time on Total Betalain Content

Figure 5 shows the effect of sonication time on total betalain content. The total betalain content first increased with an increase in sonication time and upon further increase started to decrease. Similar results have been reported by (Zimareet *al.*, 2021) [10] in their study titled "Optimization of ultrasound-assisted extraction of total phenolics and flavonoids from the leaves of Lobelia nicotianifolia and their radical scavenging potential". The number of cavitation micro-bubbles created by ultrasound increased with the duration extended. The asymmetric collapse of micro-bubbles near surfaces was also associated with micro-jets that could scour surfaces and damage substances in the solution (Vilkhuet *al.*, 2008)

[9].Therefore, the total betalain content at first increased with an increase in sonication time and decreases when the duration was extended. The pigment first increased with an increase in sonication time but slowly decreased when the duration continued to be extended. Most of the pigment in broken cells is released at the early period of extraction because ultrasound enhanced the release of those compounds into the exterior solvent (Tiwari *et al.,* 2009) [11].

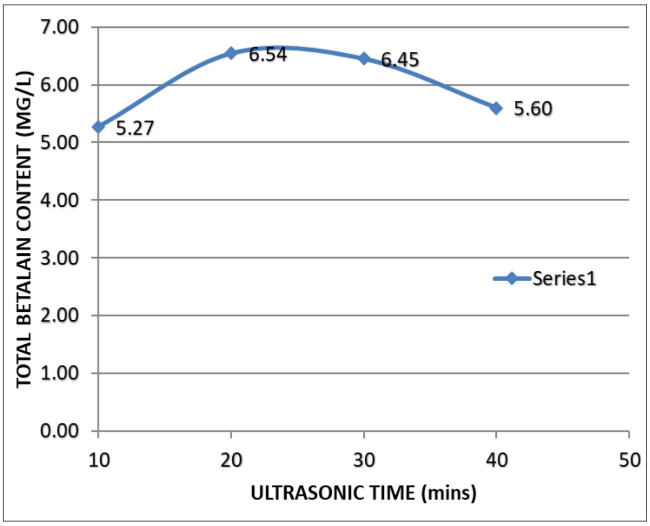

Fig 5: Effect of Ultrasonic Time on Total Betalain Content

Effect of Ultrasonic Power on Total Betalain Content

The values for Total betalain Content in the extracts varied between 4.48 – 7.08 mg/g dw. Figure 6 shows that Total Betalain Content increased up to a maximum of 7.08 mg/g dw when ultrasonic power was increased from 150 W to 200 W. A subsequent decrease in Total betalain content was observed at higher levels of ultrasonic power. It is known that an increase in the ultrasonic power supplied to the liquid medium increases the extraction performance by flavouring sonochemical effects and cavitation; however high-power values decrease cavitation in the liquid medium, prevailing agitation instead of cavitation, which results in a lower yield (Chemat F, *et al.*, 2017) [12]. Similar results were reported by Tabio-García *et al.*, 2021 [13] while studying the Optimization of the ultrasound-assisted extraction of betalains and polyphenols from *Amaranthus hypochondriacus* var. Nutrisol.

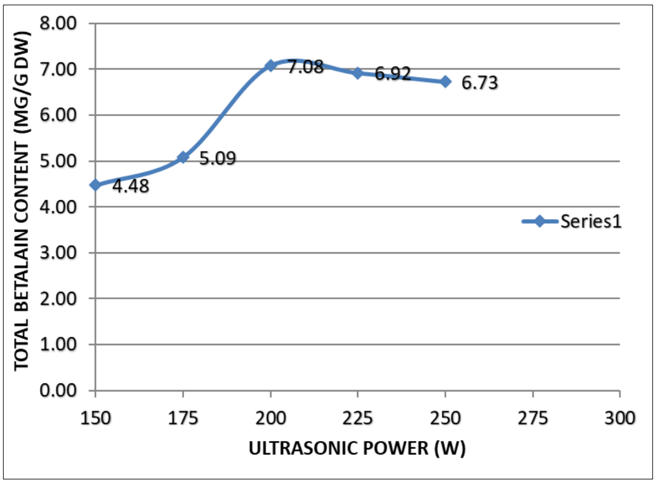

Fig. 6: Effect of ultrasonic power on total betalain content

Figure 7 shows the effect of the liquid-solid ratio on Total Betalain Content. It reveals that Total Betalain Content betacyanin increases gradually. This may attribute to the fact that as the ultrasound breaks the cell wall mechanically by cavitation shear forces, it also transfers pigments from the cell wall into the solvent. Cavitation increases the polarity of the system, including extractants, analytes, and matrix and this increases the extraction efficiency. A larger liquid-solid ratio promotes an increasing concentration gradient between the solvent and sample, as a result of which larger mass transfer between the solvent and sample occurs (Maran *et al.*, 2013) [14].

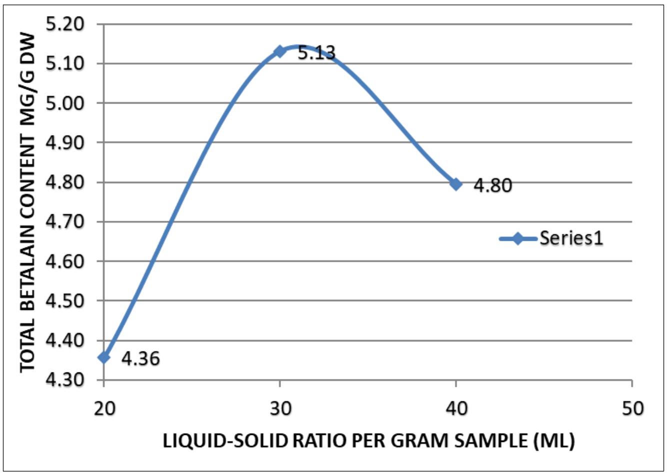

Fig 7: Effect of liquid-solid ratio on total betalain content in ultrasound assisted extraction

Conclusion

This study was conducted to understand the different extraction methods *viz* conventional, microwave-assisted extraction and ultrasound-assisted extraction by extracting the betalain pigments from the waste stalks of *Celosia cristata* and the influence of process parameters *viz*, extraction time, ultrasound power, microwave power, and the ratio of liquid to solid.

Experiments were conducted using the single-factor experiment design wherein one factor was varied at a time keeping other factors constant. Variables selected for the experiment were Temperature in case of conventional extraction; Power, Time, Liquid-Solid ratio (ml/g) in ultrasound-assisted extraction and Power, Time, Liquid-Solid ratio in ml/g in microwave-assisted extraction.

The analysis showed the betalain production for betacyanin ranged from 2.98 mg/g dw to 7.02 mg/g dw. The analysis shows that the highest betalain content observed was found to be at 4.46, 5.82, and 7.02 mg/g dw in the case of Conventional, Ultrasound-Assisted Extraction, and Microwave-Assisted Extraction respectively.

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