Advances in Research

Volume 26, Issue 2, Page 207-213, 2025; Article no.AIR.132497
ISSN: 2348-0394, NLM ID: 101666096

Ultrasound-assisted Extraction of Bioactive Compounds from Beetroot *(Beta vulgaris L.)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/air/2025/v26i21289

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/132497

Original Research Article

Received: 11/01/2025
Accepted: 13/03/2025
Published: 20/03/2025

ABSTRACT

Beetroot (Beta vulgaris L.) is a nutrient-rich vegetable known for its high concentration of bioactive compounds such as betalains, phenolic compounds, flavonoids, and carotenoids, which exhibit significant health benefits. This study investigates the potential of Ultrasound-Assisted Extraction (UAE) as a green and efficient method for enhancing the yield of these bioactive compounds from beetroot. Beetroot samples were processed to extract compounds using an aqueous solution, followed by ultrasonication and subsequent analysis. The results demonstrated that UAE significantly improved the extraction of betalains, with betacyanin and betaxanthin concentrations increasing by 16.1% and 10.9%, respectively, compared to traditional extraction methods. Phenolic compounds, flavonoids, and carotenoids also exhibited enhanced concentrations, indicating the efficacy of UAE in disrupting plant cell walls and facilitating greater solubilization of bioactives. UAE

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Cite as: *Singh, Pragati, Pranjali Chauhan, Somya Khanna, Shubhangi Srivastav, and Ekta Singh Chauhan. 2025. "Ultrasound-Assisted Extraction of Bioactive Compounds from Beetroot (Beta Vulgaris L.)". Advances in Research 26 (2):207-13. https://doi.org/10.9734/air/2025/v26i21289.*

offers a sustainable alternative to conventional extraction techniques by reducing solvent use and processing time while maintaining high extraction efficiency. The findings of this study highlight the potential of UAE to optimize the recovery of valuable compounds from beetroot, contributing to the development of functional foods and nutraceuticals. Future research could explore solvent optimization and process conditions to further increase extraction yields.

Keywords: Bioactive extract; green extraction; betaxanthin; betacyanin; functional food; ultrasonicated assisted extraction.

1. INTRODUCTION

The health benefits of fruits and vegetables are closely tied to their role as diet-enriched "functional foods" that contribute to overall health and disease prevention. Beetroot (*Beta vulgaris* L.) has emerged as a popular functional food for its health-enhancing properties. Beetroot, a nutrient-dense vegetable, is botanically categorized as “a herbaceous biennial from the Amaranthaceae-Chenopodiaceae family”, that has been appreciated for its rich nutritional content and numerous health benefits for centuries (Kohajdová et al., 2018; Thiruvengadam et al., 2024; Singh et al., 2024; Neha et al., 2018). Originating from the Mediterranean region, beetroot is now a staple in various cuisines and traditional medicine practices around the world. The edible roots of red beet contain about 9-12% of carbohydrates, 1.5-1.6% of protein, 0.1- 0.2% of total fats and 4-10 % of total sugars, 8% fibre, and essential minerals such as calcium, potassium, phosphorus, and iron (Neha et al., 2018; Chen et al., 2021; Kumar et al., 2023; Janiszewska, 2014; Zia et al., 2021). Ranked among the top ten vegetables for antioxidant properties, beetroot serves as an excellent source of phytochemical compounds like betalains, phenolic compounds, flavonoids, carotenoids, and saponins. These bioactive components offer antimicrobial, anticarcinogenic, cardioprotective, and inflammation reducing effects. Their antioxidant properties are valuable for reducing oxidative stress, decreasing oxidative reactions, and has a major impact in treating and preventing chronic diseases (Silva et al., 2018; Salamatullah et al., 2021; Bangar et al., 2022).

The extraction of bioactive compounds from beetroot has received considerable attention due to its rich array of health-promoting substances. The extraction process aims to maximize the yield and effectiveness of these compounds for optimal use in functional foods, nutraceuticals, and pharmaceuticals (Pushparaj et al., 2022; Singh et al., 2024). The extraction of bioactive compounds is affected by several factors, such as the choice of extraction method and solvent used. Traditional methods like maceration, Soxhlet extraction, and reflux extraction often require large quantities of organic solvents and extended extraction times, which can have significant environmental and safety implications. In response, research has focused on developing "green extraction" methods that minimize solvent use and reduce environmental impact (Nutter et al., 2020; Singh et al., 2024). One of the most effective non-conventional methods is Ultrasound-Assisted Extraction (UAE). UAE uses high-frequency sound waves that disrupts plant cell walls, increasing the ability of solvent to penetrate the cells and improving extraction yield. This method, which operates at frequencies above 20 kHz, is efficient, environmentally friendly, and requires relatively simple laboratory equipment, such as an ultrasonic bath. UAE is recognized for its simplicity, effectiveness, and reduced extraction time, making it a preferred technique for extracting bioactive compounds (Singh et al., 2024; Fernando et al., 2021).

2. MATERIALS AND METHODS

2.1 Plant Material

Beetroots were obtained from a local market in Banasthali Vidyapith, Rajasthan, India. The roots bulbs were washed thoroughly with running water to remove any residue of soil. It was sliced up and dried in an air-circulated oven at 75- 85◦C to complete dryness for about 18-20 hours. The dried beetroots were converted into coarse powder using electrical grinder and was later used for a bioactives extraction. Fresh to dry yield was calculated by using moisture loss method and Results were expressed as dry weight (DW).

2.2 Extraction of Bioactive Compounds

The extraction of bioactives from dried beetroots were carried out using method given by Singh et al., (2024), Nutter et al., (2020). 50g of dry sample were dissolved in 100 ml of distilled water and heated in water-bath for 2 hours at

100- 120◦C. Later, it was exposed in ultrasonic water-bath for 2 hours and then solvent was evaporated using rotary evaporator at 69rpm with bath temperature 45◦C till it was concentrated. The extracts were dried in an air circulated oven at 45-50◦C to remove the excess moisture (Mahmood et al., 2018).

The extracts were stored in an airtight container for further analysis of bioactive compounds present in it.

2.3 Estimation of Bioactive Compounds

Estimation of betalains: A spectrophotometric method, as methodologies described by Singh et al., (2024) was used to determine the betacyanin and betaxanthin content in beet greens extract by measuring their absorbances at 538 nm and 480 nm respectively.

Estimation of total phenolic compounds and flavonoids: The total phenolic content was determined using the Folin-Ciocalteu method, Flavonoids content was determined using aluminium chloride method, by following the method by Singh et al., (2024).

Estimation of Carotenoids: The determination of total carotenoids was analysed by following the procedure explained by Rosecler et al., (2009). 50 mg of the sample was mixed with the solution of diluted methanol (90:10 V/V) and HCL. This mixture was homogenized using a mini-turrax at medium speed for one minute, while kept in an ice bath and shielded from light. Later the mixture was centrifuged at 2000 rpm for 5 minutes. The supernatant was analysed using UV/VIS spectrophotometry. Absorbance was measured for chlorophyll a and chlorophyll b at 663 nm and 647 nm respectively, 529 nm for anthocyanins, and 470 nm for carotenoids. The absorbance readings were converted into concentrations (mg of total carotenoids/g dried weight) using the following formulas:

Carotenoids (µmol/mL) = {Abs470 - [17.1(Cla + Clb)] - 9.479 anthocyanins} / 119.26
Chlorophyll a (µmol/mL) = 0.01373 (Abs663) - 0.000897 (Abs529) - 0.003046 (Abs647)
Chlorophyll b (µmol/mL) = 0.02405 (Abs647) - 0.004305 (Abs529) - 0.005507 (Abs663)
Anthocyanins (µmol/mL) = Abs529 - 0.288(Abs650)

Estimation of alkaloids, saponins and tannins: Alkaloids, saponins, and tannins were determined by following procedures explained in Singh et al., (2024).

2.4 Statistical Analysis

The results were analyzed using paired t-test in MS Excel.

3. RESULTS AND DISCUSSION

Fresh to dry yield of beetroot after oven drying for 18-20 hours was 10.57%. dried beet green powder was used to prepare the aqueous extract, ethanol extract and hydromethanol extract.

Betalains are water-soluble pigments found in beetroot (Beta vulgaris) that are primarily responsible for its vibrant red color. These pigments are divided into two main types: betacyanin, which produce red to violet hues, and betaxanthins, which give yellow to orange shades. Research has shown that betalains possess strong antioxidant properties, contributing to various health benefits, including anti-inflammatory effects and potential protection against chronic diseases (Silva et al., 2018). Beetroot contains 8.28 mg/100g of betaxanthin and 6.26 mg/100g of betacyanin. In comparison, its aqueous extract exhibits slightly higher concentrations, with 9.18 mg/100g of betaxanthin and 7.27 mg/100g of betacyanin as shown in Fig. 1. Upon analysing the data using t-test, it was found that the levels of betaxanthin and betacyanin in beetroot aqueous extracts were highly significant ($P < 0.001$), compared to their levels in beetroot. Some studies suggest that using organic solvents, such as alcohols, might be necessary to achieve a better yield of betalains (Fernando et al., 2021; Fathordoobady et al., 2016; Celli & Brooks, 2017).

3.1 Estimation of Total Phenolic Compounds and Flavonoids

The antioxidant, antimicrobial, and anti-inflammatory properties of flavonoids and phenolic compounds present in beetroot make it a valuable functional food (Kohajdová et al., 2018). As indicated in Fig. 1, beetroot contains 7.89 mg/g of total phenolic compounds and 6.76 mg/g of flavonoids. These values align with the findings by Rosecler et al., (2009) In comparison, the beetroot aqueous extract exhibits enhanced concentrations, with 12.46 mg/g of total phenolic compounds and 9.25 mg/g of flavonoids. similar findings were reported by Borjan et al., (2022) with diluted methanol as a solvent during ultrasonicated assisted extraction (Rosecler et

al., 2009; Borjan et al., 2022). Statistical analysis conducted using paired t-test revealed that the levels of both the total phenolic compounds and flavonoids in beetroot aqueous extract were highly significant ($P < 0.001$), compared to their levels in beetroot.

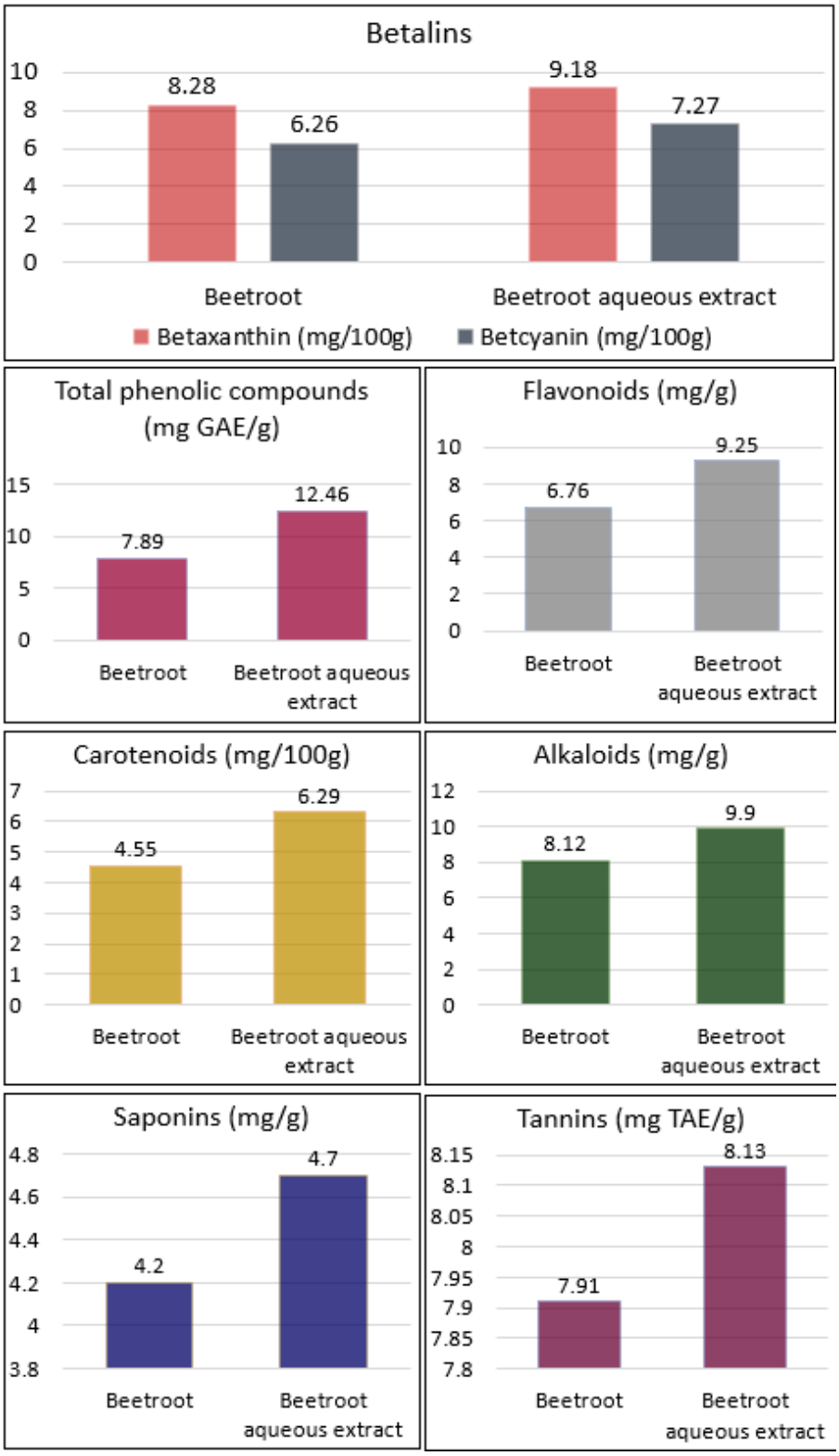

Fig. 1. Bioactive analysis of beetroot and beetroot aqueous extract Estimation of betalains

3.2 Estimation of Carotenoids

Carotenoids are organic lipophilic pigments found in plants, ranging in color from yellow to red and playing crucial roles in photosynthesis and photoprotection. Carotenoids are not only important for plants but also hold significance for human health, with provitamin A activity and potential anti-cancer properties (Doukani et al., 2022). Beetroot contains 4.55 mg/100g of carotenoids, while its aqueous extract exhibits an increased concentration of 6.29 mg/100g as shown in Fig. 1. The levels of carotenoids in beetroot aqueous extract were highly significant ($P < 0.001$), compared to its levels in beetroot. Similar results have been observed in organic beet roots (Rosecler et al., 2009). The carotenoid content in beetroot surpasses that found in many fruits, as reported by Sant'ana et al. (2007) and is similar to the levels identified in various carrot varieties, as noted by Alasalvar et al. (2005); Sant'ana et al., (1998); Alasalvar et al., (2012).

3.3 Estimation of Alkaloids, Saponins and Tannins

Beetroot (Beta vulgaris) is rich in various bioactive compounds, including alkaloids, tannins, and saponins, which contribute significantly to its health benefits. Alkaloids in beetroot are linked to antioxidant properties that may help prevent chronic diseases. Tannins, a type of polyphenolic compound, are known for their ability to bind and precipitate proteins, aiding in digestive health and potentially exhibiting anti-inflammatory effects. Saponins, another class of compounds found in beetroot, are recognized for their cholesterol-lowering effects and their role in enhancing immune function (Nwankwo & Bamidele, 2012; Kumari & Solanki, 2019).

The analysis of bioactive compounds in beetroot and its aqueous extract revealed notable variations. As shown in Fig. 1, beetroot itself contains 8.12 mg/g of alkaloids, 4.2 mg/g saponins, and 7.91 mg TAE/g of tannins. In comparison, the beetroot aqueous extract shows enhanced concentrations, with 9.9 mg/g of alkaloids, 4.7 mg/g saponins, and 8.13 mg TAE/g of tannins. The levels of alkaloids, saponins, and tannins in beetroot aqueous extract were highly significant ($P < 0.001$), compared to their levels in beetroot.

4. CONCLUSION

The finding of the study highlights the enormous potential of beetroot as a source of bioactives such as betalins, carotenoids, phenolic compounds, flavonoids, etc,. The enhanced levels of these bioactives in the beetroot aqueous extract obtained using the Ultrasound-Assisted Extraction (UAE) method demonstrate the efficiency of UAE as a green and efficient extraction methodology. The efficacy of UAE in this study demonstrates its potential for widespread use in extracting bioactive, providing a sustainable strategy while maximising the health advantages of natural goods such as beetroot as a valuable source for the development of functional foods, nutraceuticals and pharmaceuticals.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors acknowledge the use of AI-assisted tools, including ChatGPT, to enhance the flow, language, and readability of the manuscript. These tools were employed solely for linguistic improvements and did not contribute to the conceptualization, analysis, or interpretation of the research findings. The scientific content, originality, and conclusions remain the sole responsibility of the authors.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Mr. Vimal Jain for their technical assistance when performing the experiments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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