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COMPARATIVE ANALYSIS OF BIOACTIVE COMPOUND EXTRACTION FROM BEET GREENS: A STUDY ON THE INFLUENCE OF SOLVENTS USED IN ULTRASONIC ASSISTED EXTRACTION

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Keywords:

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ABSTRACT: This study investigates the extraction of bioactive compounds from *Beet vulgaris* L. greens, a member of the Chenopodiaceae family known for its high antioxidant capacity. Despite being a horticultural product with substantial underused biomass, beet greens are traditionally discarded as waste, representing a lost opportunity for valuable bioactive components. This research focuses on the extraction of such bioactive compounds using different solvents (ethanol, methanol, and water) by Ultrasound Assisted Extraction (UAE). The results reveal that dil. methanol (1:1 v/v) extract named as hydromethanol extract, is particularly effective in extracting betalains, demonstrating higher betaxanthin content compared to ethanol and aqueous extracts. Aqueous extract excels in extracting total phenolics, while hydromethanol extract outperforms in extracting flavonoids. Ethanol extract exhibits superior efficiency in extracting alkaloids, while hydromethanol extract stands out for saponin and tannin extraction. This study underscores the significance of solvent selection in optimizing the extraction of specific bioactive compounds from beet greens. The findings contribute valuable insights into developing sustainable and efficient extraction processes for utilizing beet greens as a rich source of bioactive compounds in various industries, including food and pharmaceuticals. Further considerations such as cost, safety, and practical applications are crucial for selecting the most suitable solvent for specific purposes.

INTRODUCTION: *Beta vulgaris* L., a member of the Chenopodiaceae family, is one of the ten vegetables with the highest antioxidant capacity [1, 2, 3]. It acts as an excellent dietary supplement due to its abundance in vitamins, minerals, and other nutrients along with its special phytochemicals, which offer a number of therapeutic benefits [4, 5]. Although it is one of the horticultural products that produce a significant amount of underused biomass [3].

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In general, beet greens (stems and leaves) account for approximately 50% of the total plant and are traditionally disposed of as waste, implying that half of the biomass will be discarded after harvest before reaching consumers [6, 7, 8]. However, Beet greens are a valuable source of bioactive components such as pigments (betacyanin, and betaxanthies) and phytochemicals (polyphenols, chlorophylls, carotenoids) [9].

Almost all of these bioactive components have beneficial properties, including antimicrobial, anti-inflammatory, anticarcinogenic, and cardio-protective properties [10, 11, 12]. These bioactive substances are useful as food additives because of their antioxidant capabilities [13]. Antioxidants are crucial for reducing oxidative stress, facilitating a decrease in oxidative reactions, and are significant for the treatment and prevention of chronic illnesses [5]. Artificial antioxidants have become popular due to their durability and accessibility. But with increasing concern over the safety of products made using synthetic chemicals, which are thought to promote adverse health consequences Exploring naturally available bioactive compounds from such by- products as a replacement to synthetic substances, is becoming more and more popular nowadays [13].

Extraction of these bioactive compounds for being used as ingredients for functional foods is a better and safe alternative for waste management of the waste generated from the food processing industries [14]. The extraction of bioactive compounds is influenced by several variables, including the extraction method, and the extraction solvent [15]. The methods can be divided into conventional and non-traditional categories.

In conventional methods, such as Maceration, Soxhlet extraction, Reflex extraction, *etc*., organic solvents are used in larger quantities with a longer time of extraction. However, the use of larger quantities of such solvents has its own environmental impacts as well as safety aspects linked with the handling of such substances [8]. Thus, various research studies were conducted to find extraction methods where less or no organic solvents were used to minimize its impact on the environment [15]. Advanced non-conventional methods such as Microwave assisted (MAE), Ultrasound assisted (UAE), Enzyme assisted (EAE), etc., extraction methods were developed. These processes were also known as “Green Extraction” which are more effective, efficient, and cleaner than the traditional conventional methods. Over time, these methods have been improved to enhance the extraction efficiency and reduce the extraction time with lesser or no number of solvents used [16].

Ultrasound Assisted Extraction (UAE) uses ultrasound waves are sound waves with high frequency beyond human hearing capability [16]. In the process of UAE, plant cell walls are disrupted using ultrasound at frequencies higher than 20 kHz, which aids the solvent's capacity to permeate the cells and results in a better extraction yield. As it uses basic laboratory equipment like an ultrasonic bath, UAE is recognised as one of the simplest extraction procedures [7, 17]. In this study we are using ultrasound assisted extraction (UAE) method for the extraction of bioactive compounds from the beet greens, by using different solvents such as ethanol, dil. methanol and water.

MATERIALS AND METHODS:

Plant Material: Beet plants were obtained from a local market in Banasthali Vidyapith, Rajasthan, India. The beet greens were separated from the roots by cutting it 1 cm above the bulbs, then it was washed thoroughly with running water to remove any residue of soil.

It was dried in an air-circulated oven at 80◦C to complete dryness for about 18-20 hours. The dried beet greens 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).

Extraction of Bioactive Compounds: The extraction of bioactives from beet greens were carried out by slightly modified method given by Mehmood *et al.,* (2018) [19]. Three extracts were prepared using distilled water, ethanol and hydromethanol named aqueous extract, ethanol extract and hydromethanol extract, respectively.

Aqueous Extract (A.E.): 50g of dry sample were dissolved in 100ml of distilled water; the solution was heated in water-bath at 100◦C for 2 hours.

Ethanol Extract (E.E.): 50g of dried sample was dissolved in 100ml of ethanol; the solution was let sit for 48 hours in room temperature (25-30°C).

Hydromethanol extract (H.M.E.): 50g of dried sample was dissolved in 100ml of diluted methanol (1:1 v/v); the solution was let sit for 48 hours in room temperature (25-30◦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 a air circulated oven at 45-50°C to remove the excess moisture. The extracts were stored in an airtight container for further analysis of bioactive compounds present in it.

Estimation of Betalains Content: Determination of the betalains was carried out by the method given by Abdo *et al.*, (2022) [19]. A spectrophotometric method was used to determine the betacyanin and betaxanthin content in beet greens extract by measuring their absorbances at 353 nm and 438 nm respectively. The following equation was used foe the calculation.

$$\text{Betacyanin / Betaxanthin (mg/L)} = A \times DF \times MW \times V\ \varepsilon L \quad (1)$$

In this equation, (A) represents the absorbance of the samples, (DF) represents the dilution factor for the extract, (MW) represents the molecular weight of the pigments (550 g/mol for betacyanins and 308 g/mol for betaxanthins), and (V) represents the volume of the extract. For betacyanins and betaxanthins, the extinction coefficients in water are 60,000 and 48,000, respectively, and the path length of the cuvette is 1 cm.

Estimation of the Total Phenolic Content: The diluted methanolic extract (0.5 ml of 1:10 g/ml), gallic acid (a standard phenolic compound), and folincoicalteu reagent (5 ml, 1:10 diluted with water) were mixed together, and then neutralised with an aqueous solution of sodium carbonate (4 ml 1M) was added. Upon allowing the mixture to stand for a period of 15 minutes, a spectrophotometer was used to determine the amount of total phenols at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50 v/v). The total phenolic content was expressed in terms of gallic acid equivalent (GAE mg/ml of dry mass) [20].

Estimation of the Total Flavonoids: The flavonoid content was determined by the aluminium chloride method. 1 mL of each of the beet greens extract was mixed with 4 mL of distilled water and 0.3 mL of 5% of sodium nitrite and it was incubated for 5 minutes then 0.3 mL of 10% aluminium chloride was added, and the samples were incubated for another 6 minutes.

Later, 2 mL of sodium hydroxide (1 mol/L) was added, and the volume was completed upto 10 mL of distilled water. The mixture was then incubated for another 15 min, and the absorbance was measured at 510 nm. The total flavonoid value is expressed as µg/mL of catechin [21].

Estimation of Alkaloids: To determine the alkaloid contents, 5 g of the beet greens extract was mixed with 200 mL of 10% acetic acid in ethanol, the mixture was then covered and rested for 4 hours. Later the mixture was filtered, and the filtrate was evaporated on a water bath till concentrated upto the quarter part of the original volume. Concentrated NH4OH was added drop wise to the filtrate until precipitation was complete. The solution was rested and allowed to settle to collect the precipitate which was later washed with dilute NH4OH and filtered. The alkaloids are collected as the form of residue, which was dried and weighed [22].

Estimation of Total Saponins: To calculate the total saponin, 20 g of the sample powder was added to with 100 mL of diluted ethanol (1:5 distilled water v/v), and the mixture was heated at approximately 55°C over a hot water bath for four hours while being stirred continuously. 200 mL of diluted ethanol (1:5 distilled water v/v) was used to obtain the residue after filtering the mixture. The combined extracts were concentrated to 40 mL over a water bath that had been heated to about 90 °C. 20 mL of diethyl ether was added after pouring the concentrate into a 250 mL separating funnel, and the mixture was then vigorously stirred. The aqueous layer was recovered, whereas the ether layer was discarded. The purifying process was carried out again. 60 mL of n-butanol were added. On the combined n-butanol extracts, two separate washes with 10 mL of 5% aqueous sodium chloride were performed. The saponin concentration was calculated after the remaining solution was evaporated until concentrated in a water bath and then dried further in the oven to a constant weight [22].

Estimation of Tannins: The Folin-Ciocalteu method was used for estimation of tannins. 0.1 ml of extract was dissolved in 7.5 ml of distilled water before it was mixed with 0.5 ml of the Folin reagent. Later 1 ml of 35% sodium carbonate was added, and 10 ml of distilled water was used to dilute it. After thoroughly shaking, the mixture was left at room temperature for 30 minutes. Gallic acid reference standard solutions at concentrations of 20, 40, 60, 80, and 100 µg/ml were created. Using a UV/Visible spectrophotometer, absorbance for test and standard solutions was measured against the blank at 725 nm. The amount of tannin was calculated as mg of GAE/g of extract [20].

RESULTS: Fresh to dry yield of beet greens after oven drying for 18-20 hours was 6.78%.

Dried beet green powder was used to prepare the aqueous extract, ethanol extract and hydromethanol extract.

FIG. 1: BEET STALKS GREENS (FRESH AND DRY)

Later aqueous extract, ethanol extract and hydromethanol extract of beet greens were studied to determine the presence of bioactive compounds.

Betalains: Beet greens were found to be rich source of betalins, as **Fig. 2** indicates that the highest content of betaxanthin was 9.18 mg/100g which was observed in hydromethanol extract followed by ethanol and aqueous extract with 5.29 mg/100g and 5.24 mg/100 g respectively. The betacyanin content of hydromethanol extract was highest with 7.87 mg/100 g followed by aqueous and ethanol extract with 7.27 mg/ 100g and 6.9 mg/100grespectively.

Total Phenolic Content: as shown in **Fig. 3** the total phenolic content of beet greens was observed highest in the aqueous extract which was 4.7 GAE mg/ml followed by hydro methanol extract with 3.64 GAE mg/ml; the least amount of total phenolic content was observed in the ethanol extract with 0.121 GAE mg/ml.

Flavonoids: As shown in **Fig. 4** the flavonoid content in beet greens hydromethanol extract was highest with 9.29 mg/g followed by Aqueous extract with 7.69 mg/g and ethanol extract with 7.29 mg/g.

Alkaloids: as indicated in **Fig. 5** the alkaloid content was observed to be highest in ethanol extract with 12.71 mg/g almost twice as the aqueous extract with 6.32 mg/g; alkaloids content in hydromethanol extract were 7.98 mg/g.

Saponin: as shown in **Fig. 6** the concentration of saponin in beet greens was similar in aqueous and ethanol extract with 0.45% but highest saponin content was observed in hydromethanol extract with 0.52%.

Tannins: as **Fig. 7** indicates that the highest content of tannins was 275 mg/L which was observed in hydromethanol extract followed by ethanol and aqueous extract with 270 mg/L and 261 mg/L respectively.

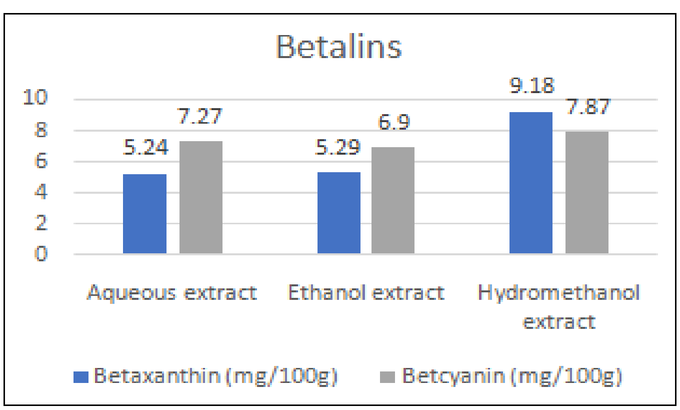

FIG. 2: BETALINS CONTENT IN AQUEOUS, ETHANOL AND HYDROMETHANOL EXTRACT OF BEET GREENS

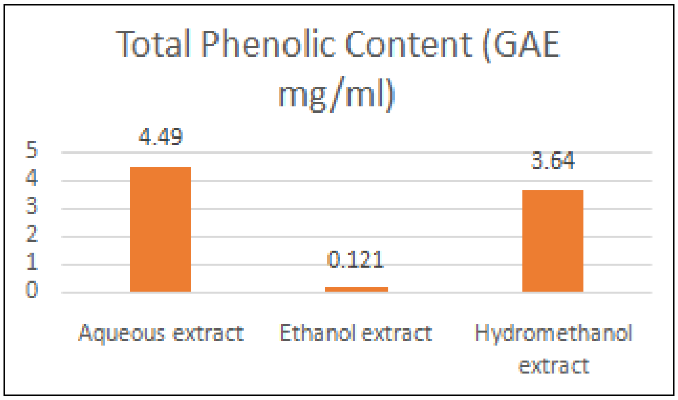

FIG. 3: TOTAL PHENOLIC CONTENT IN AQUEOUS, ETHANOL AND HYDROMETHANOL EXTRACT OF BEET GREENS

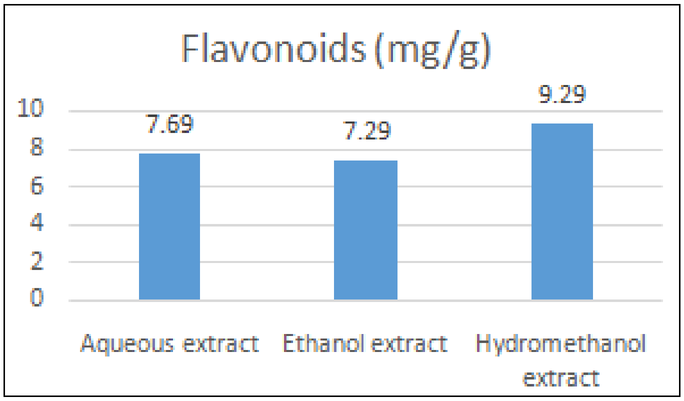

FIG. 4: FLAVONOIDS CONTENT IN AQUEOUS, ETHANOL AND HYDROMETHANOL EXTRACT OF BEET GREENS

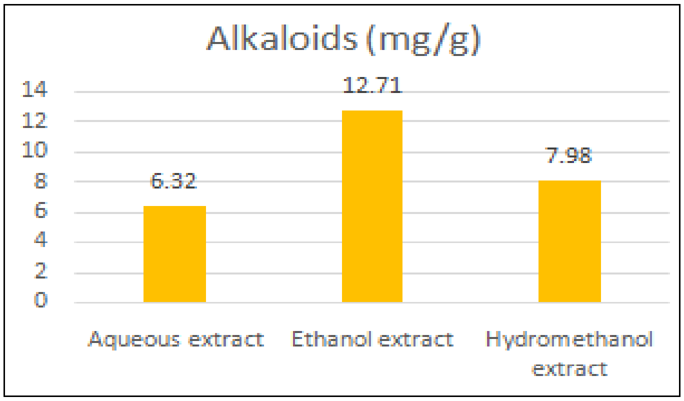

FIG. 5: ALKALOIDS CONTENT IN AQUEOUS, ETHANOL AND HYDROMETHANOL EXTRACT OF BEET GREENS

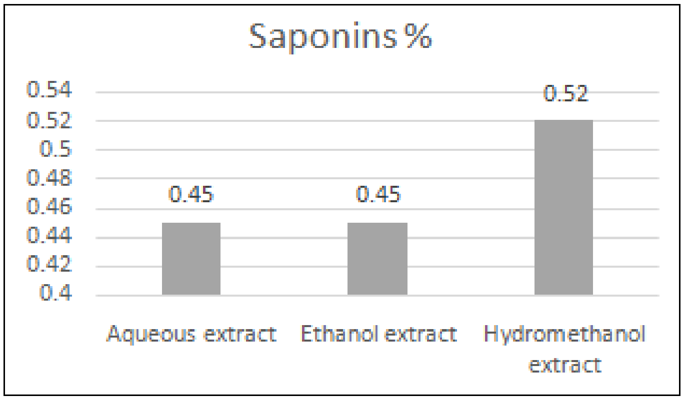

FIG. 6: SAPONIN CONTENT IN AQUEOUS, ETHANOL AND HYDROMETHANOL EXTRACT OF BEET GREENS

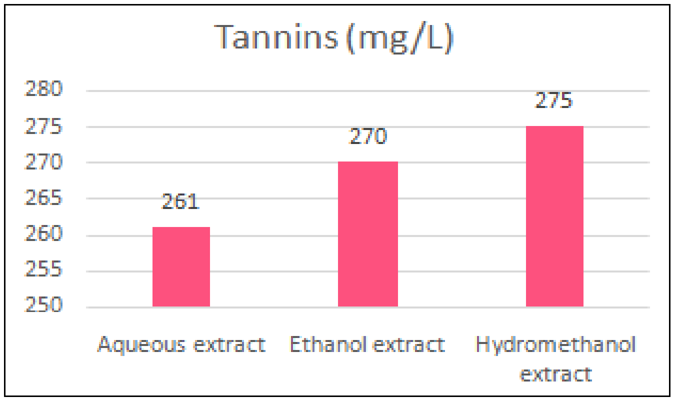

FIG. 7: TANNINS CONTENT IN AQUEOUS, ETHANOL AND HYDROMETHANOL EXTRACT OF BEET GREENS

CONCLUSIONS: In conclusion, *Beta vulgaris* L., particularly its underutilized beet greens, emerged as a valuable source of bioactive compounds with significant therapeutic benefits. This study explored the potential of beet greens, traditionally discarded as waste, for their rich content in pigments, polyphenols, and other bioactive components. The utilization of such by-products aligns with the growing interest in natural alternatives to synthetic antioxidants, considering the safety concerns associated with artificial additives. The extraction of bioactive compounds from beet greens was undertaken using the environmentally friendly Ultrasound Assisted Extraction (UAE) method, showcasing its effectiveness in comparison to conventional methods. The choice of solvents ethanol, methanol, and water further influenced the extraction efficiency and the type of bioactive compounds obtained.

Results indicated that the UAE method, particularly with hydromethanol, proved highly successful in extracting betalains, phenolics, flavonoids, alkaloids, saponins, and tannins from beet greens. The concentration of these bioactive compounds varied among the different extracts, showcasing the potential for tailoring extraction processes to obtain specific bioactive components. The findings underscore the transformative potential of beet greens in contributing to functional foods and nutraceuticals, with implications for industries seeking sustainable alternatives. This research not only enhances our understanding of the bioactive profile of beet greens but also highlights the importance of "Green Extraction" methods for maximizing yield while minimizing environmental impact. This approach aligns with the broader trend of utilizing plant by-products for value addition, waste reduction, and the development of health-promoting products.

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