The Research of Medical Science Review

Received: 23 September, 2024
Accepted: 26 October, 2024
Published: 16 November, 2024

ISSN: 3007-1208 | 3007-1216
Volume 2, Issue 3, 2024

ANTIOXIDANT ACTIVITY OF BETA VULGARIS (RED BEETROOT), ITS TOTAL PHENOLIC CONTENT AND PRODUCT DEVELOPMENT

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ABSTRACT
Beetroot and other colorful root vegetables exhibit strong antioxidant activity due to the presence of polyphenol compounds. Epidemiological research has demonstrated a negative correlation between fruit and vegetable diet and the risk of developing human cancer due to the presence of antioxidant phenolic compounds. This study was conducted to evaluate the antioxidant activity of red beetroot, its total phenolic content and product development. Three types of beetroot products were developed including beetroot chips, beetroot sauce and muffins (beetroot colored). The antioxidant and proximate analysis of the beetroot products was conducted. Beetroot powder antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) technique and a UV-Vis spectrophotometer. The results of proximate analysis showed that beetroot is particularly high in fiber and sugars. The antioxidant analysis of beetroot powder showed that it is rich in phytochemicals and thus exhibits strong antioxidant potential. The results of the study indicate that red beetroot contains significant amount of polyphenols due to which it exhibits anti-inflammatory, hepatoprotective, anti-cancer properties. Hence, it can be used as a functional food with powerful antioxidant and anti-inflammatory properties.
Keywords: *beetroot, antioxidant, phytochemicals, anti-inflammatory, anti-cancer*

INTRODUCTION

Plant foods are rich in natural antioxidants including lipid-soluble vitamin E, carotenoids, water-soluble vitamin C, and phenolic compounds. Red beetroot, is a useful plant-based raw material that has been shown to provide health benefits for people. Beetroot is a biennial plant, meaning it completes its life cycle over two years, though it is usually harvested in its first year for consumption (Hamedi & Honarvar, 2019).

Beetroot abundantly provide minerals such as manganese, sodium, potassium, magnesium, iron, and copper. Additionally, it also contains antioxidants, vitamins A, C, and B, fiber, and natural colors. Red beetroot also provides high quantity of phenol molecules, which have anti-oxidant qualities. These vibrant root vegetables offer protection against some types of cancer, including colon cancer, and heart disease (Kavalcová et al., 2015).

Beetroot belongs to the Amaranthaceae family, which includes other well-known plants like spinach, chard, and quinoa. It is a biennial plant, meaning it completes its life cycle over two years, though it is usually harvested in its first year for consumption. Betacyanins, which are red pigments, give beets their distinctive hue. Now a days, red beetroots are becoming a more significant vegetable because of its numerous beneficial physiological and nutritional qualities (Székely & Máté, 2022).

Sliced beetroot can be baked or fried into beet chips, offering a crunchy and healthy alternative to potato chips. These chips retain the beetroot's natural sweetness and are often seasoned with salt, pepper, or herbs (Mudgal et al., 2022).

It is a great source of dietary fiber, which supports gut health and facilitates digestion. The fiber content promotes the growth of good gut flora, eases constipation, and helps control bowel motions. Betalacains, which are strong antioxidants found in beetroot, improve liver function and aid in the body's detoxification process. These chemicals support the body's removal of toxic substances and their neutralization (Milton-Laskibar et al., 2021).

Phytochemicals, which are bioactive compounds found in plants, also have antioxidant properties. These include flavonoids, carotenoids, and polyphenols. They are abundant in fruits, vegetables, and whole grains and are responsible for many of the health benefits associated with a diet rich in plant-based foods (Guldiken et al., 2016).

Epidemiological research has demonstrated a negative correlation between fruit and vegetable diet and the risk of developing human cancer due to the presence of antioxidant phenolic compounds. These phenolic substances support the body's first and second defenses against oxidative stress. Among the phenolic substances flavonoids and isoflavonoids are particularly encouraging options for preventing cancer (Birt et al., 2001).

Vegetables and fruits are essential components of a balanced diet and can help avoid a number of illnesses. The beetroot (Beta vulgaris L.) has gained popularity recently as a possible functional food. The potent antioxidant found in beetroot is betalains, primarily betanin. Betalains lower the risk of liver and kidney damage, cardiovascular and cerebrovascular disorders, and certain types of cancer (Chen et al., 2021).

Pickling is a popular way to preserve beetroot. Slices of beetroot are typically soaked in a vinegar-based brine with spices, resulting in a tangy, slightly sweet condiment that pairs well with sandwiches, salads, and meats. It can also be fermented, a process that enhances its probiotic content and creates a tangy, slightly fizzy product. Fermented beetroot, or beet kvass, is a traditional drink in some Eastern European countries, known for its potential health benefits (Khandekar et al., 2020).

Researchers have always been drawn to the beet plant's enticing color and nutrient-dense roots to investigate and create its edible products. As a perishable food, beetroot may get dehydrated, which could result in a quantitative increase in its mineral content—particularly in iron and calcium—due to a decrease in water mass (Dhawan et al., 2019).

Material and Methods

The aim of the study was to evaluate the antioxidant and product development potential of beetroot.

Product development

Three different types of beetroot products were developed with beetroot as main ingredient. These products include: beetroot chips, beet sauce and muffins (beetroot colored).

Development of beetroot chips

Half a kilogram of beetroot was washed and cleaned thoroughly. The beetroot was sliced into thin, even rounds. The beetroot slices were marinated in a bowl with five grams of salt, five grams of black pepper, ten milliliters of olive oil, five grams of mixed herbs, and five grams of oregano for fifteen minutes. The air fryer was preheated to 200 degrees Celsius (400 degrees Fahrenheit). The marinated beetroot slices were arranged in a single layer in the air fryer and were air fried for ten to fifteen minutes, or until they were crispy and golden brown (Frîncu et al., 2022).

Development of Beet sauce

100 milliliters of beetroot juice was extracted from fresh beetroot. 5 milliliters of apple cider vinegar, 5 milliliters of sweet chili sauce, and 5 milliliters of soy sauce were added to the beetroot juice. 5 grams of toasted and ground sesame seeds were sprinkled over the mixture. 5 grams of dried oregano, salt, and pepper were added to taste. All ingredients were mixed thoroughly until well combined (Kryzhova et al., 2021).

Development of Muffins (beetroot colored)

250 grams of all-purpose flour, 100 grams of granulated sugar, 10 grams of baking powder, 2

grams of salt, and 10 grams of beetroot powder were combined in a bowl. In a separate bowl, 1 egg was whisked together with 60 grams of melted butter and 250 milliliters of milk. The wet ingredients were gradually added to the dry ingredients and mixed until just combined. The muffin tin was placed in a preheated oven at [insert desired temperature] and baked for 18-20 minutes, or until a toothpick inserted into the center came out clean. The muffins were allowed to cool in the tin for a few minutes before being transferred to a wire rack to cool completely (Bajraktari et al., 2024).

Antioxidant analysis of beetroot products

Beetroot powder antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) technique and a UV-Vis spectrophotometer. This approach assesses antioxidants' ability to remove free radicals using a change in absorbance.

Preparation of DPPH Solution

Powdered DPPH was diluted in methanol to provide a stock solution of 0.1 mM DPPH in accordance with (Marinova & Batchvarov, 2011) .

Preparation of sample

The beetroot powder extract was prepared by immersing 5 mL of 80% methanol in 0.5 g of dehydrated and powdered beetroot and stirring in a water bath at 100 rpm for 2 hours. Following the extraction process, the mixture was filtered to yield a transparent extract suitable for testing.

Preparation of reagents:

Prepare Folin-Ciocalteu reagent, sodium carbonate solution, and a standard gallic acid solution. The preparation of Folin-Ciocalteu reagent was carried out in accordance with (Ainsworth & Gillespie, 2007).

Extraction of phenolic compounds:

In accordance with , 5 grams of powdered beetroot were accurately weighed and underwent extraction with 50 milliliters of 70% methanol. The mixture was stirred for an hour and a half to make sure enough phenolic components were extracted into the solvent.

Mix Sample with Reagent:

Combine the sample extract with the Folin-Ciocalteu reagent.

Add Sodium Carbonate Solution:

Add sodium carbonate solution to the mixture.

Incubating the Mixture

Allow the mixture to incubate at room temperature.

Measuring Absorbance Level

The absorbance of the solution was measured at a wavelength of 765 nm with a UV-VIS spectrophotometer. Create a standard curve using the gallic acid standard solutions. Determine the phenolic content of the sample by comparing its absorbance to the standard curve. The assessment provided the necessary information for measuring the phenolic content. Three experiments were conducted to assess precision and dependability (Sirivibulkovit et al., 2018)

Nutritional Analysis Of Beetroot Products

The nutritional parameters which were assessed in this research were moisture, protein, fat, ash, moisture, and fibre. Accurately determining the concentrations of carbohydrates, proteins, lipids, and minerals in various food samples depends on widely used techniques including carbonated, Kjeldahl, Soxhlet, and ash analyses utilising a muffle furnace (Odebunmi et al., 2010).

Results and Discussion

Antioxidant activity of beetroot:

In the present study it was observed that the estimated concentration of polyphenols when the absorbance is 1.23 is approximately 100.32 (in the units consistent with calibration, typically µg/mL or mg/mL). The computed quantity of about 100.32 (µg/mL or mg/mL) indicates a considerable amount of polyphenols in the sample.

Table 3.1: Concentration, Absorbance, And Percentage Inhibition Of Samples

Concentration (µL)	Absorbance	Percentage inhibition
25	0.786	60.6%
50	0.589	70.5%
751	0.462	76.8%
100	0.368	81.5%

Figure 3.1: Concentration vs. Absorbance Curve for Polyphenols in Red Beetroot

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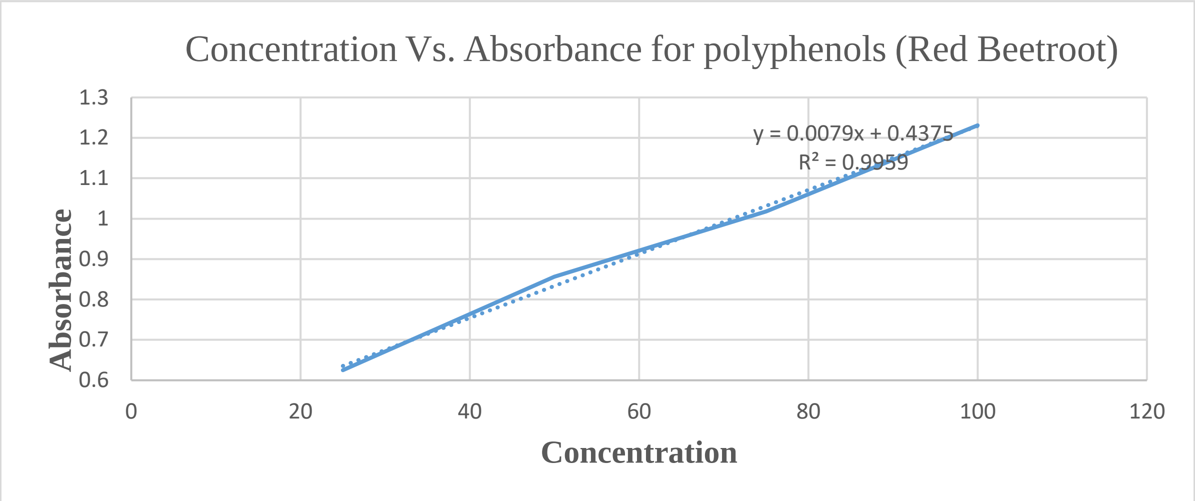

standard calibration chart for polyphenols in beetroot is crucial for precisely estimating the phenolic content in samples, providing a framework for quantification, validation, and comparison with existing literature.

Using the calibration equation: y=0.0079x+0.437 setting y=1.23, rearranging the equation to solve for x (the concentration of polyphenols):

1.23=0.0079x+0.4375

x=0.079÷0.7925≈100.32

Antioxidant analysis of beetroot powder showed good intensity of antioxidant activity which was investigated by DPPH as shown in figure. The graph evaluated the absorbance value of beetroot powder which was 0.368 as compared to the concentration 100 µL, which indicates good intensity of antioxidant activity. This was also evident by Percentage inhibition which was found to be 81.5% at 100 µL.

A higher polyphenol concentration often indicates more antioxidant activity, which can give a variety of health benefits such as anti-inflammatory properties, improved cardiovascular health, and improved exercise performance. The result is also supported by (Kavalcová et al., 2015) in which the total polyphenol content of beetroot was evaluated using a compound DPPH$^{\cdot}$ (2.2-diphenyl-1-picrylhydrazyl) at 515.6 nm in spectrophotometer. The results indicate that beetroot exhibit high antioxidant activity.

Antioxidant activity of beetroot powder with different concentrations was observed

From 60% to 80% with varying concentrations of solvent. Comparing to the previous studies kumar et al. found 85.2% inhibition by DPPH method.

Singh et al. measured the %age inhibition by FRAP method and the results were 78.5%.

Ravichandran et al. measured the percentage inhibition value by ORAC method results were significantly higher in this case as the value obtained was 92.1%.

Kanner et al. measured the percentage inhibition value by TEAC method, the results obtained were the highest among all studies and methods that was 95.6%.

The antioxidant content measured was in proximity to the studies done before. Percentage inhibition values varies depending upon the method of calculating, different varieties of beetroot, processing conditions (including drying temperature and storage conditions), extraction solvent and the concentration of extract. The study conducted showed that red beetroot is rich in antioxidants as the measured content was significantly higher. Beetroot's high antioxidant content may provide health benefits as cell protection, anti –inflammation and anti-cancer food.

Total phenolic content(TPC) measured with different concentrations after making a standard calibration curve was 100.32mg GAE/g. Comparing to the previous studies done on beetroot phenolic content was present in a range of 100 to 300 mg GAE/mg of beetroot.

Wang et al. observed the TPC of 123.4mg GAE/mg. Lee et al. calculated total phenolic content and flavonoids to be 145.6mg GAE/g. Zhang et al. did a study on beetroot flavonoids content, the results were in close proximity with the study conducted. Zhang et al. measured 108.2mg GAE/g total phenolic content.

Kumar et al. did the research on beetroot phenolic activity, and concluded result were 120.1mg GAE/g. The study conducted by Singh et al. on total phenolic content of red beetroot showed the value as 130.8mg GAE/g.

Difference between the conducted study and previous studies varies because of the extraction methods, different solvents, different beetroot varieties and different processing conditions that can significantly impact total phenolic content of beetroot.

Table 3.2: Nutrient Composition of Beetroot Chips, Sauce, Muffins, and Powder (per 100g)

The proximate analyses of beetroot products are given in table:

Nutrients	Beetroot chips Quantity/100g	Beetroot sauce Quantity /100g	Muffins Quantity /100g	Beetroot powder Quantity /100g
Protein content	4.2%±2.1	0.6%±2	5.2%±2.1	13.4%± 5
Carbohydrate content	4.9%±2.2	7.61%±2	49.7%±2	70.3%±10
Fat content	5.8%±3	0.3%±0.2	18.7%±3	1.1%±1
Fiber content	6.1%±2	0.8%±1	1.6%±0.8	6.3%±5
Ash content	4.7%±1	1.5%±2g	2.43%±1	3.4%±2
Moisture content	7.9%±3	91.1%±2	21.6%±5	1.2%±1

From the results of proximate analyses, we can conclude that the beetroot is rich in carbohydrate content, as all the products of beetroot contain carbohydrate in high amount. Additionally, it can also be observed that the beetroot products also contain protein and fat in significant amount.

Thus, beetroot can be used as a food additive to increase the nutritional value of a food. This result is supported by a study conducted by (Dhawan et al., 2019) in which the proximate and antioxidant analysis of beetroot powder was conducted and the results showed that beetroot flour contains a good amount of micronutrients and phytochemicals and can be used as functional foods.

A study conducted by (Chen et al., 2021) highlights beetroot's potential health benefits, particularly in combating oxidative stress, supporting cancer treatment, managing chronic diseases, and improving physical performance. Proximate analysis of beetroot further reveals its significant macronutrient content, reinforcing its role as a valuable nutritional source.

Another study conducted in Nigeria concludes that beetroot is a valuable source of carbohydrates, protein, fiber, and key minerals necessary for the body's optimal function. This suggests that beetroot holds significant nutritional potential and should be considered for consumption (Shuaibu et al., 2021).

The results of proximate analysis reveal that the protein content in the beetroot powder is highest as compared to the other processed products. Hence, it reveals that the processing conditions can affect the nutritional profile of beetroot.

The results in the study are supported by a study conducted by (Paciulli et al., 2016) in which the effects of high-hydrostatic pressure (HHP) treatments (650 MPa for 3, 7, 15, and 30 minutes) on beetroot slices were explored as an alternative to blanching (90°C for 7 minutes). While HHP only partially inactivated polyphenol oxidase (PPO) and peroxidase (POD), it retained more nutrients compared to blanching. Total phenolic content and antioxidant capacity were similar in both treatments.

Another study was conducted by (Bianchi et al., 2021) in which Pasteurization decreased betalain content but did not affect antioxidant activity, while storage led to color changes and further reductions in both betalains and antioxidant activity. Thus, the study revealed that the study revealed that processing and storage affect the nutritional quality of beetroot-based products.

Conclusion:

The present study was conducted to evaluate the antioxidant activity of red beetroot (beta vulgaris) and its total phenolic content through product development. The results of the proximate analysis show that the beetroot is a good source of carbohydrates, proteins and other macronutrients. Also the analysis conducted to evaluate the antioxidant activity showed that the beetroot powder exhibit strong antioxidant activity. Thus, the results suggest that the beetroot can be used as a functional ingredient as its antioxidant potential can be utilized to prevent or cure several diseases. Moreover, the beetroot contains polyphenols due to which it exhibit anti-inflammatory, hepatoprotective, anti-cancer properties. As a plant based food stuff; beetroot consumption will not only provide health benefits to the people but also will not pose any risk.

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