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Department of Chemistry

Spectrophotometric Extraction Study of Betanin from *Beta Vulgaris*

A graduation research project
Submitted to the Department of Chemistry in partial fulfillment of the requirements for the completion of the degree of Bachelor of Science in Chemistry

By

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Acknowledgment

In the name of Allah, the most Gracious and Most Merciful First and for most, I praise Allah, the almighty for providing me this opportunity and granting me the strength to complete my project after all the challenges and difficulties.

I would like to express my deepest gratitude to ***Dr. Mashael A. Alghamdi.***

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FINALY, I wish to express my deepest thanks to my family without whom nothing of this work have been accomplished.

Abstract

Beet root (*Beta vulgaris*) is a plant that is rich of many natural organic biomolecules. Betalains, which is one of these compounds, are found to have many biological benefits. Many studies were conducted to extract these biomolecules from different sources including beetroot. Spectrophotometer was used in this study to extract betalains. Different solid / liquid ratios and different solvent mixture ratios were tested to find the best extraction condition. Based on the absorbance readings the ratio 1:5 of solid/liquid ratio was the best ratio to measure the content of betanin and the 1:1 ratio of (water : ethanol) ratio was the best solvent mixture ratio to extract and measure the concentration of betanin at 535 nm. In addition, thermal and photo stability and sensitivity of betanin was also investigated, and the results proved that the betanin structure influenced by the high temperature and light.

الخلاصه

الشمندر هو نبات غني بالعديد من الجزيئات الحيوية العضوية الطبيعية. البيتالين، أحد هذي المركبات، هي مركبات وجدت أن لها العديد من الفوائد البيولوجية. وقد أجريت العديد من الدراسات لاستخراج هذه الجزيئات الحيوية من مصادر مختلفة بما في ذلك الشمندر. تم استخدام مقياس الطيف الضوئي في هذه الدراسة لاستخلاص البيتالين. تم اختبار نسب المواد الصلبة/السائلة المختلفة ونسب خليط المذيبات المختلفة للعثور على أفضل حالة للاستخلاص. بناءً على قراءات الامتصاص، كانت نسبة 1:5 من نسبة الصلبة إلى السائل هي أفضل نسبة لقياس محتوى البيتانين وكانت نسبة 1:1 من نسبة (الماء: الإيثانول) هي أفضل نسبة خليط مذيب لاستخلاص وقياس التركيز. البيتانين عند 535 نانومتر. بالإضافة إلى ذلك، تم أيضاً دراسة الثبات الحراري والضوئي وحساسية البيتانين، وأثبتت النتائج أن تركيب البيتانين يتأثر بارتفاع درجة الحرارة والضوء.

List of Abreviations

Abbreviation	Scientific name
UV	Ultraviolet
Vis	Visible
DW	Distilled water
Bc	Betanin concentration
A	Absorption
DF	Dilution factor
M_w	Molar weight
ε	Extension coefficient
L	Length of cuvette
RT	Room temperature
IR	Inferred spectroscopy
h	Hour

Chapter1

Introduction

1.1. Background of the study

Beta vulgaris (beet root) is a species of flowering plant withinside the subfamily Batoidea of the personal family Amaranthaceous. Economically, it`s miles the most important crop of the big order Caryophyllales [1]. It has several cultivar groups: the sugar beet, of greatest importance to offer table sugar; the idea vegetable known as the beetroot or garden beet; the leaf vegetable known as chard or spinach beet or silver beet; and mangelwurzel, that could be a fodder crop. All cultivars, no matter their quite different morphologies, fall into the subspecies *Beta vulgaris* subsp. vulgaris. The wild ancestor of the cultivated beets is the sea beet (*Beta vulgaris*) subsp [1,2]. The leaves and roots of beets are packed with nutrition, together with antioxidants that fight molecular damage and reduce the threat of coronary heart illness. They`re one of the few vegetables that consist of Betalains, a powerful antioxidant that gives beets their colorful color [3].

Betalains from red beetroot are natural pigments, which embody each yellow or orange betaxanthins or red violet betacyanins. However, betalains are quite sensitive in the direction of heat, pH, light, and oxygen, which ends up withinside the horrific stability at some point of processing and storage. Therefore, it`s miles crucial to realize the influences of the processing techniques on betalains [1,2].

1.2. Objective and scope

Current trends indicate consumer preferences for the consummation of natural products. For this reason, the purpose of this project consists of the conduction of an extraction study of betalains derivatives from *Beta vulgaris*, as a relevant source, and also to study the thermal stability of the extract via different operatory conditions.

1.3. Arrangement of project

The developed project includes five chapters:

Chapter 1 is reserved to the background and objective of this project.

Chapter 2 includes the literature review section, in which we have talked about the following grand lines:

-In the first part, Betalains which cover the following titles: Betalains classification, Sources of Betalains, Advantages of betalains derivatives, Antibacterial activity, Application of betalains derivatives, Dye-Sanitized solar cell application, Food Pigment application.

-In the second part, Extraction of natural compounds which cover the following titles: Conventional methods, Nonconventional methods, Ultrasound extraction method, Micro-wave assisted extraction, Super-critical fluid extraction method

-In the third part, *Beta vulgaris*: Source of extracted Betanin which cover the following titles: Generality, Betanin content

-Chapter 3 and chapter 4 correspond respectively to the experimental and results and discussion sections.

-Chapter 5 includes the project conclusion and recommendations for further study.

Chapter2

Literature Review

2.1. An overview on betalains

Betalains are attractive natural pigments with potent antioxidant activity. The call "betalains" comes from the Latin call of the not unusual place beet (*Beta vulgaris*), from which betalains had been first extracted [2].

2.1.1. Potential sources of betalains

The potential sources of betalains include fruits, roots, leaves, peels, stems, and seeds with red or violet color are considered. The contents of betalains are related to the type of raw materials, extraction techniques, and extraction conditions. Table 1 exposes the primary sources of betalains [4].

Table 1: Primary sources of betalains.

Sources	Betalains (mg/100g)	Betacyanins (mg/100g)	Betaxanthins (mg/100g)
Amaranthus spp	0..7.00-20.93	159.09	-
B. spectabilis		456.00	116.00
B. rubra	13.81-677.00	124.18-142.00	19.16-535.00
O. ficus indica	41.54-2252	159-1655	126-686
H. polyrhizus	-	17.64-82.79	-
S. stellatus	479.30	-	-
O. joconostle	-	92.00	-
O. engelmannii	20160.00	-	-
B. vulgaris	-	27.50-649.00	16.30-446.70
A. sessilis	-	-	7450.00
C. quinoa Willd	-	96.47	201.01
S. fruticosa	12,990.00	-	-

2.1.2. Chemical classification of betalains

Betalains own a uniform structural feature this is derived from betalamic acid (Fig. 1), collectively with an intensive R1 or R2, wherein the substituents may be a hydrogen or radical. The variant withinside the substituent agencies originate from numerous origins of pigments and impacts their balance and hue. Two predominant paperwork consist of yellow betaxanthins and crimson, violet betacyanins. Beetroots have about 75−95% betacyanins and five−25% betaxanthins (Fig. 1). More than 80% of pigments from crimson beetroot encompass betacyanins, namely, betanin and isobetanin, an isomer of betanin [5].

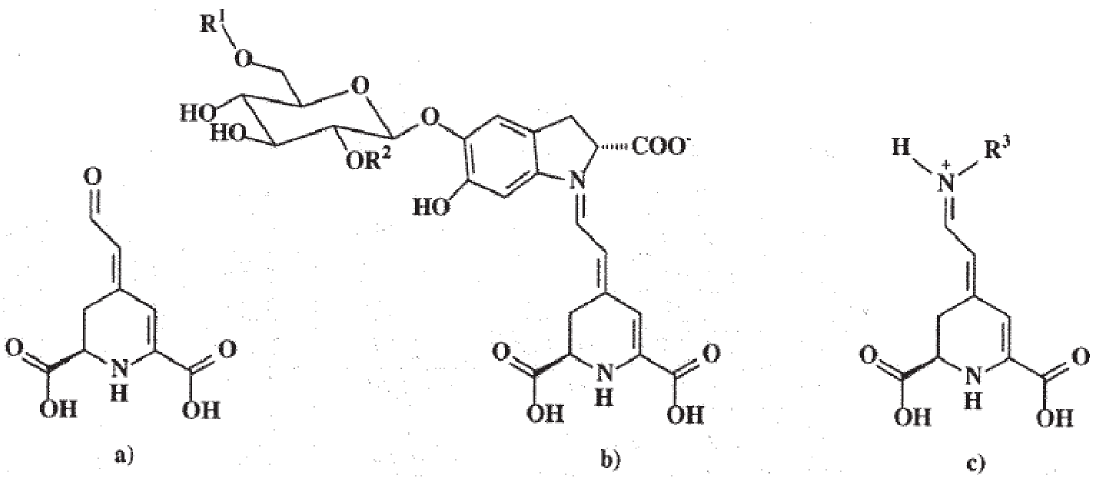

Figure 1: Betalamic acid (a), betacyanins (b) and betaxanthins (c) [5].

2.1.3. Chromatic aspects of betalains

The natural red beet extracts are likely a mixture of several pigments. The betalain pigments are characterized by a maximum absorbance at about 535 nm (λ_{max}) for the red purple betacyanins (betanin with λ_{max} = 535 nm and betanidin with λ_{max} = 542 nm) and near 480 nm for the yellow betaxanthins (indicaxanthin, the common betaxanthin found in red beet root with λ_{max} = 482 nm); for the betalamic acid λ_{max} = 424 nm [6].

2.1.4. The spectrophotometric quantification of betanin content

The spectrophotometric quantification of betanin content in studied extracts has been determined using optical spectroscopy, by measuring the absorbance at λ 535 nm, using an UV-VIS spectrophotometer. The obtained absorbances were used to calculate the betanin concentration of each sample according to following equation [7]:

$$Bc = \frac{A * DF * M_w * 1000}{\varepsilon * L}$$

Bc = betanin content (mg·L-1)

A = absorbtion at (535nm)

DF = the dilution factor

Mw = molecular weight of betanin (550 g/mol)

ε = molar extinction coefficient of betanin (60000 L . mol- . cm-1 in H2O)

L = the pathlength of the cuvette (1cm)

2.1.5. Application domains of betalains derivatives

Betalains are attractive natural pigments with potent antioxidant activity. They form a reliable alternative to synthetic dyes and are applied in multidisciplinary fields. We expose in this section the more important application domains [8].

2.1.5.1 Dye-Sanitized solar cell application

A dye-sensitized sun based totally on betalains pigments extracted from pink beet roots was described. A photoanode changed into manufactured from nanocrystalline TiO_2 on obvious conductive glass, dealt with ethanolic HCl answer, then sensitized with uncooked beet extract. The betanin-sensitized movie while hired in a dye-sensitized sun mobileular gave a most photocurrent of 2.42 mA/cm^2 and open-circuit photovoltage of 0.44 V withinside the presence of methoxy propionitrile containing I−/I3− redox mediator [6].

2.1.5.2. Food Pigment application

Due to the synthetic red colorants' adverse effects on human health as a food additive, there is an increased interest in searching for natural pigments, e.g., betalains. Beetroot, as a food pigment, is used for coloring some dairy food products, such as yogurts, candies, jellies, chicken frankfurters, sausages, and ice cream. Also, beetroot as a dietary supplement or food additive could enhance sensory attributes, nutrition values, and bioactivities. Currently, betalains are widely employed in the food industry as an additive or a natural dye, and they exhibit in vitro and in vivo antioxidant ability mainly due to the presence of betacyanins. In addition, betalains can be applied in the packaging of food with natural polymers [9, 10, 11, 12].

2.1.5.3. Antioxidant Activity

Red beetroot is renowned for its antioxidant activity since it contains betalains that are important for maintaining and regulating the redox process. The excellent antioxidant activity of betalains is due to their unique molecular structures, which is reflected by their capability to donate hydrogen to reactive species. Betalains are also significant antiradical ingredients since they are efficient ROS scavengers [13, 14].

2.1.5.4. Anti-inflammatory Activity

Betanin, the most abundant betalain in beetroot, was found to possess anti-inflammatory activity through inhibition of cyclooxygenase, hypochlorous acid scavenging, and oxidants produced by neutrophils during the inflammation reaction [13, 14].

2.1.5.5. Antimicrobial Activity

Red beetroot pomace can effectively reduce the infection area of Salmonella typhimurium,
Bacillus cereus, and Staphylococcus aureus. The main compounds of red beetroot pomace are betalains, which could suppress Gram-negative bacteria infection (e.g., Escherichia coli, Citrobacter f reundii, Pseudomonas aeruginosa, Citrobacter youngae, etc.). It was found that betalains had antibacterial activity, because they could affect structure, function and permeability of microbial cell membrane, which eventually resulted in death of the microorganism [13, 14].

2.2. Extraction of betalains

2.2.2. Solid liquid extraction process

Solid–liquid extraction is the separation of a solid solute from a mixture of solids by dissolving it in a liquid phase. Basically, there are three components: solid solute, insoluble solids and solvent. In most cases, the diffusion of intra-particle soluble component(s) controls the extraction rate. Therefore, the process is often called as diffusion extraction. Solid–liquid extraction is widely used in food (e.g. extraction of sugar from sugar cane or sugar beet; isolation of vegetable oils from different seeds) and pharmaceutical industries (e.g. Extraction of active components from medicinal plants), and in hydrometallurgy (e.g. leaching of metal ions from ores). The solvent added to the dry raw material is partly taken up by the solid material and the soluble ingredients are instantaneously dissolved. Often all the solute is already dissolved [4, 10].

2.2.3. Conventional extraction method

The conventional extraction approaches like maceration and Soxhlet employ some organic
solvents with or without heat treatment. Red beetroot pigments are usually extracted by physical pressing of the sliced red beetroots or centrifugation of ground roots followed by blanching.

Aqueous extraction of shredded beetroots has also been used. The addition of ethanol or methanol to water is generally necessary to thoroughly extract the pigments in the presence or absence of acidification or heat treatment, especially for the improved extraction yield. A recent study evaluated the optimal extraction conditions (ethanol concentration and time) to recover betalains in red beetroot (*Beta vulgaris L.)*. The results showed that betalains can be extracted from beetroot using water and ethanol. The optimal extraction time was 43 min, and the yields were 31.03%, 45.01%, and 35.34% for betacyanins, betaxanthins, and total betalains, respectively. It was suggested that aqueous ethanol medium is appropriate for maximal recovery of beetroot betalains instead of ethanol or water as a single solvent [4, 10].

2.2.4. Nonconventional extraction Methods

Currently, some nonconventional techniques that have been used for extraction of red beetroot pigments are summarized in Table 2. The pretreatments have been proposed to improve extraction rate of betalains, including cryogenic freezing, aqueous two-phase extraction, gamma irradiation, pulsed electric field, ultrasound-assisted extraction, microwave-assisted extraction, and membrane processing. These extraction processes have advantages as alternative environmentally friendly procedures for extraction of betalains over conventional procedures, because they can decrease extraction time, posttreatment wastewater, and solvent-energy consumption [3, 4, 15, 16].

Table 2 : Different extraction techniques for betalains [3].

source of betalain	extraction method	extraction results
Red beetroot betalains	Aqueous two-phase extraction	• Differential partitioning of betalains and sugars was achieved in aqueous two-phase extraction at a higher tie line (34%), wherein 70−75% of betalains partitions to the top phase and 80−90% of sugars present in the beet partitions to the bottom phase.
Red beetroot betanin	Aqueous two-phase extraction and gel permeation chromatography	• The molar concentration of betanin was 16.5×10^{-6} mol/L, and the molar concentration of indicaxanthin was 14.0×10^{-6} mol/L. • Betanin can be used as a reducing agent to synthesize silver-betanin core−shell triangular nanodisks. • The synthesis substance exhibited antibacterial activity against *E. coli* MTCC-450 and *S. aureus* MTCC-3160.
Red beetroot betalains	Ultrasound-assisted extraction	• Ultrasound (37 and 52 °C, 165 W, 25 kHz, 90 min) rendered higher betalains and higher antioxidant activity than in the conventional methods. • Ultrasound-assisted extraction method could yield 4.20 and 2.80 mg/g of betacyanins and betaxanthins, respectively. By contrast, the conventional extraction methods yielded 3.84 mg/g of betacyanins and 2.65 mg/g betaxanthins (orbital shaker), 3.46 mg/g of betacyanins and 2.59 mg/g betaxanthins (metabolic shaker), respectively.
Red beetroot extract	Ultrasound-assisted extraction with β-cyclodextrin	• Application of 5% β-cyclodextrin aqueous solution showed the highest content of betanin of 2.243 mg), total phenolic compounds of 20.03 mg/g, and also had the highest DPPH inhibitory activity of 59.87%. • β-cyclodextrin-assisted extraction significantly increased the extraction of betanin and enhanced antiradical activity. • The extracted samples exhibited a high antiradical activity as well as enhanced stability of betanin in beverage and gummy candy models during the different storage conditions.
Red beetroot betalains	Pulsed electric field	• The use of pulsed electric field at the intensity of 4.38 kV/cm increased the yield of betanin and vulgaxanthin extraction from beetroot cylinders by 329% and 244%, compared with the control samples.
Red beetroot betalains	Microwave-assisted extraction	• The combination of 400 W/100% duty cycle and 800 W/50% duty cycle for 100 s resulted in the highest amount of betanines (128.68 and 122.90 mg of pigment/100 g of freeze-dried red beet), whereas the combination of 400 W and 100% duty cycle at 140 and 150 s conferred the highest amount of betaxanthins (101.41 and 100.29 mg of pigment/100 g of freeze-dried red beet). • When 0.04 mol/L ascorbic acid was added to the solvent prior to a two-step microwave-assisted extraction process, the highest amount of betanines was 187.67 mg/100 g red beet at 130 s/100 s and the greatest amount detected (125.43 mg/100 g red beet) under the same treatment. • The yield of betalains obtained by microwave-assisted extraction was 5-fold greater than the conventional extraction yield.
Red beetroot betalains	Cryogenic freezing	• Cryogenic freezing (20 s) and thawing (one cycle) led to the extraction of more pigments than the electric method. The higher mineral content of the immersion solution of the freeze−thawed tissue was 88.4 mg/L in comparison with those of the electrically treated (43.3 mg/L) and untreated (7.0 mg/L) tissues.
Red beetroot betanin	Gamma irradiation	• The increase in gamma irradiation doses up to 10.0 kGy led to a rise in both the batanin concentration as well as ionic components. The application of gamma irradiation tended to create a more open tissue architecture, which facilitated diffusion during solid−liquid extraction, resulting in the increased diffusion coefficient of betanin (from 0.302×10^{-9} to 0.463×10^{-9} m^2/s) and ionic components (from 0.248×10^{-9} to 0.455×10^{-9} m^2/s). The equilibrium concentration of betanin using gamma irradiation (10.0 kGy) was 7.11 mg/100 mL, while the equilibrium concentration of betanin with control was 5.58 mg/100 mL. • The exposure to gamma-irradiation pretreatment increased cell wall permeabilization, and caused loss of turgor pressure, which could increase the extractability of betanin from red beetroot.
Red beetroot betalains	Membrane technology	• Total betalain content was increased up to 46%. • Microfiltration of beet juice could remove 99% turbidity and achieve the stable flux with subsequent filtrations. • The loose reverse osmosis membrane could effectively separate betalains with 98% rejection. • Up to 96% salts and 47% of dissolved solids were separated from betalains on concentration and diafiltration.

Chapter3

Methodology

3.1. Materials

3.1.1. Chemicals

Ethanol was provided by Sigma-Aldrich and used in different dilutions.

3.1.2. Beetroot (*Beta vulgaris*) preparation

The beetroot (*Beta vulgaris*) was purchased from a local grocery store in Riyadh, Saudi Arabia. The beetroots were cleaned and cut into small pieces after removed the peel. A fresh pulp was used after grinding it in a mortar (Fig. 2). About 50 g of beetroot were used in this study.

Figure 2. The pulp of beetroot grounded in a mortar.

3.2. Methods

3.2.1. Spectrophotometric Extraction of betalains

20 g of beetroot was prepared and grinded. Then 200 ml of distilled water (DW) was added to make (1:5) (g:ml) mixture. The mixture was kept for 30 min on a magnetic stirrer at room temperature. The solution was finally filtered using a filter paper to remove any impurities (Fig. 3).

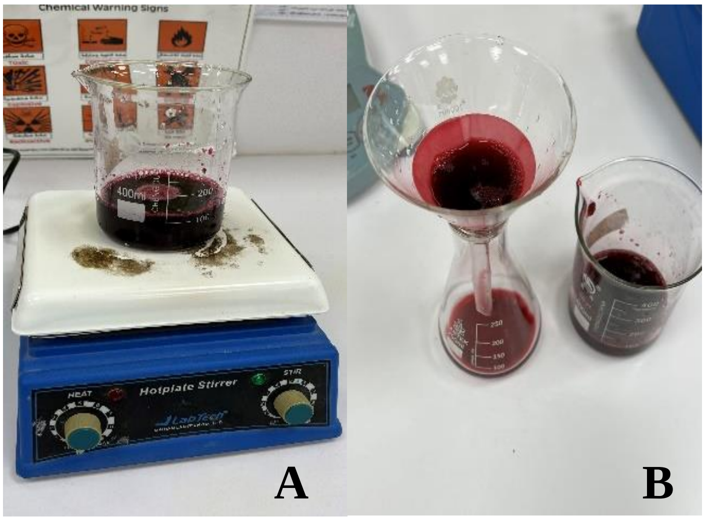

Figure 3. A) The mixture of beetroot and DW on a magnetic stirrer. B) filtering the solution through filter paper.

To determine the optimal wavelength for extracting betalains, after filtering the solution, the sample was taken in a cuvette (in triplicate) to measure the absorbance at different wavelengths (400-640 nm) by UV-VIS spectrophotometer (APEL-PD-303) (Fig. 4). The measuring takes place after zeroing the machine with blank (DW). Then the measured absorbances at different wavelengths were plotted to determine the optimal wavelength for extraction.

Figure 4. UV-VIS spectrophotometer.

3.2.2. Determination of total betanin content (Bc)

To calculate the betanin content we used the following equation. Each measurement was established in triplicate, and the average was used to calculate the betanin content.

$$Bc = \frac{A * DF * M_w * 1000}{\varepsilon * L}$$

Bc = betanin content (mg·L-1)

A = absorption at (520-550 nm)

DF = the dilution factor

M_w = molecular weight of betanin (550 g/mol)

ε = molar extinction coefficient of betanin (60000 L . mol- . cm-1 in H2O)

L = the pathlength of the cuvette (1cm)

The quantity of the Bc was measured in mg/L as Castellar and his colleagues described in his paper [17].

3.2.2.1 Effect of solid liquid ratio

In this section, distilled water has been used in all experiments as an extraction solvent. Experiments occurred at room temperature. Different solid to liquid ratios have been tested, in triplicate (1:5, 1:8, and 1:10).

3.2.2.2 Determination of total betanin content (Bc) in different solvent mixture

Based on the results of the previous section, we fixed the ratio solid to liquid to (1:5). To evaluate the effect of the solvent mixture on the extraction efficiency, the solvent ratios used in this study were (1:1, 1:4, and 4:1) (DW : Ethanol). Then the absorbances were recorded and the Bc were calculated.

3.2.3. Thermal stability of betanin

To test the thermal stability of betanin, the Bc was measured at room temperature (24°C) and at 75°C at different durations (ranged from 0 to 5 hours) for different wavelengths (520-550 nm) (Fig. 5).

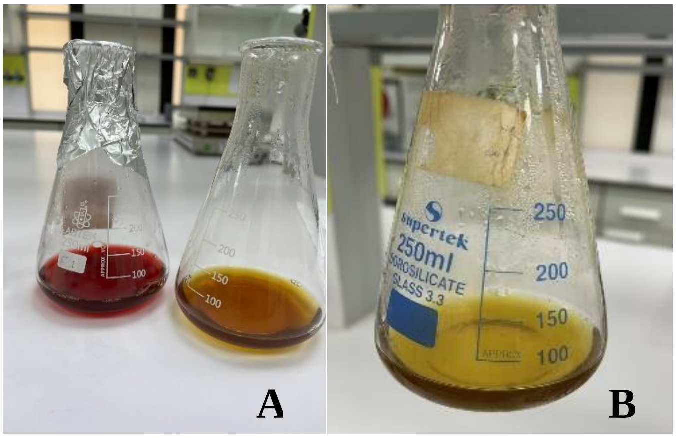

Figure 5. A) The samples at RT (to the left) and after placing it in 75°C water bath (to the right). B) The sample at 75°C after 2 h.

3.2.4. Photostability of betanin

To test the photostability of betanin, the Bc was measured at room temperature (24°C) and different duration (0, 1h, 3h, 24 h, 48h, 72h) for different wavelengths (520-550 nm), with and without exposure to light. Foil was used to prevent the exposure to light (Fig.6).

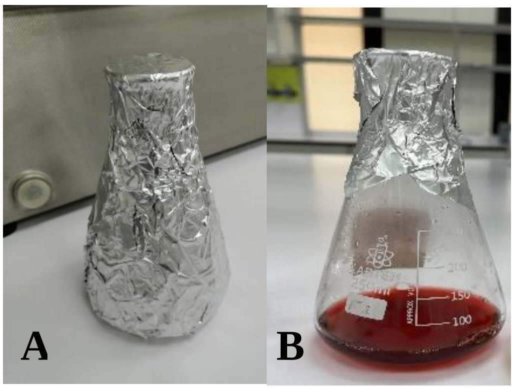

Figure 6. Samples placed at room temperature. A) Covered sample and B) Sample exposed to light.

Chapter4

Results & discussion

4.1. Spectrophotometric Extraction of betalains

Figure 7 shows the visible absorption spectra of the aqueous extract of betalains. The spectrum shows an increase in the recorded absorbance values till obtaining a maximum around 530-540 nm. Above this wavelength, a decrease in the absorbance values was recorded, and that agrees with previous published data [18].

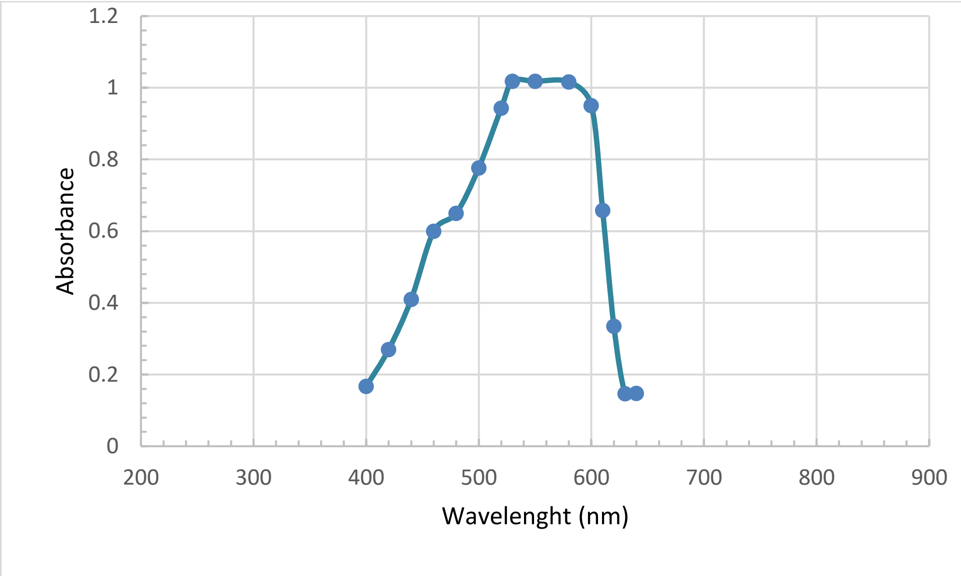

Figure 7. Visible light absorption spectra of betalains.

4.2. Optimization of betanin extraction

The quantification of the betanin content from the different extraction samples has been established using the previously mentioned equation [7].

$$Bc = \frac{A * DF * M_w * 1000}{\varepsilon * L}$$

4.2.1. Effect of solid liquid ratio

As shown in figure 8, for the different absorbances (530-535-540-550 nm), the higher betanin content has been registered for the solid liquid ratio (1g: 5 mL). For this reason, solid liquid ratio has been kept to (1:5) for the following sections.

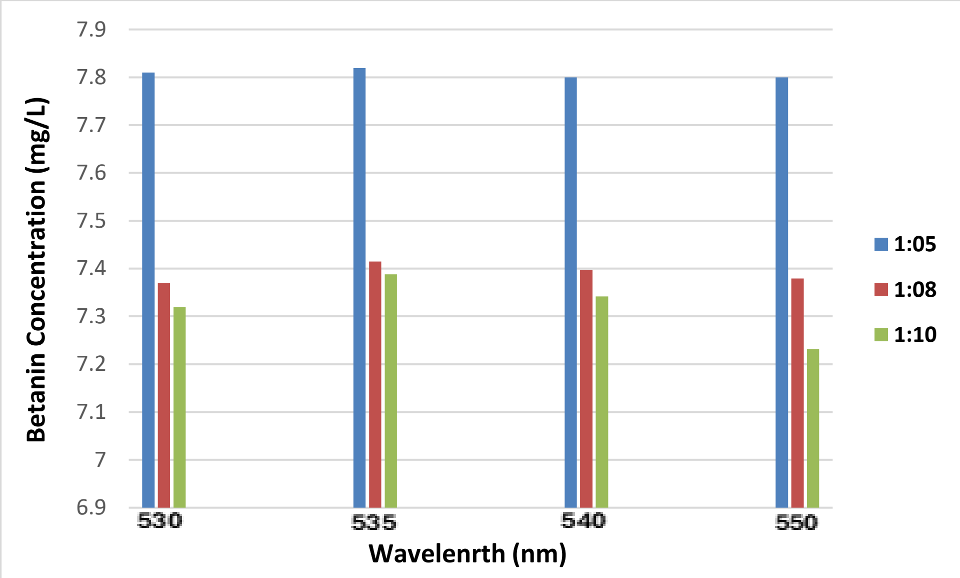

Figure 8. Bc measured in different solid liquid ratios 1:5 (blue), 1:8 (Red), and 1:10 (green) at different wavelengths (530 – 550 nm) from left to right.

4.2.2. Determination of Bc in different solvent mixture

Ethanol has been known as an efficient betalains extraction solvent [3,4]. For this reason, we have tested in this part different ratios of DW : ethanol (1:1, 1:4, and 4:1) for the extraction of betalains. The results are shown in the following figure 9. The Bc was quantified at different wavelengths (530-535-540-550 nm). The highest content of betanin was found for all different wavelengths in the 1:1 ratio which was in the range of (9-10 mg/L). Overall, the presence of ethanol in any ratio in the solvent mixture enhanced the extraction ability of the betanin [4].

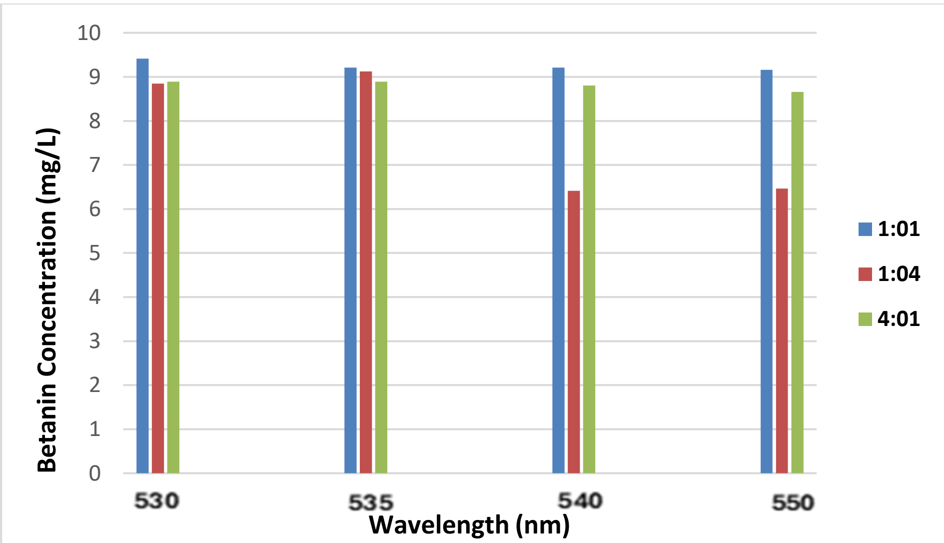

Figure 9. Bc measured in different (DW : ethanol) ratios 1:1 (blue), 1:4 (Red), and 4:1 (green) at different wavelengths (530 – 550 nm) from left to right.

4.3. Thermal stability of betanin

The wavelength 535 nm was recorded in previous studies to measure the highest concentration of betanin, so we measured the absorbance for this part of project at it [3, 4, 19]. Figure 10 shows the decrease in betanin concentration with time at 535 nm.

The concentration of betanin decreased gradually in the flask that was placed at 75°C (Fig. 10 (A)), whereas the other flask that was left at RT showed an unexpected increase after the first hour (Fig. 10 (B)). The best explanation for that is because of the presence of other compounds that resulted from the degradation of betanin (Fig. 11) [20], the improper incubation of the solutions, or insufficient broken equipment. The high temperature seems to play a critical role in degrading the betanin rapidly based on our results and that agrees with previous studies [7].

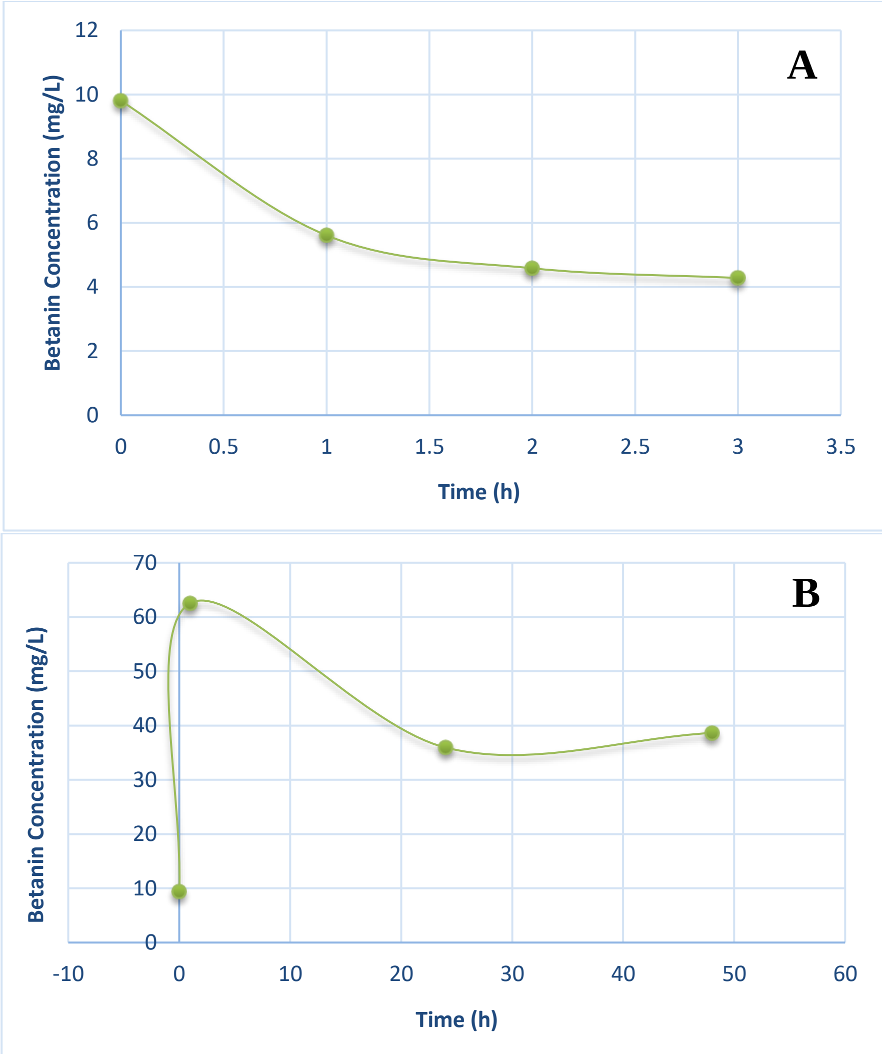

Figure 10. Bc was measured at a different time to study thermal stability. A) at a high temperature (75°C). B) at RT.

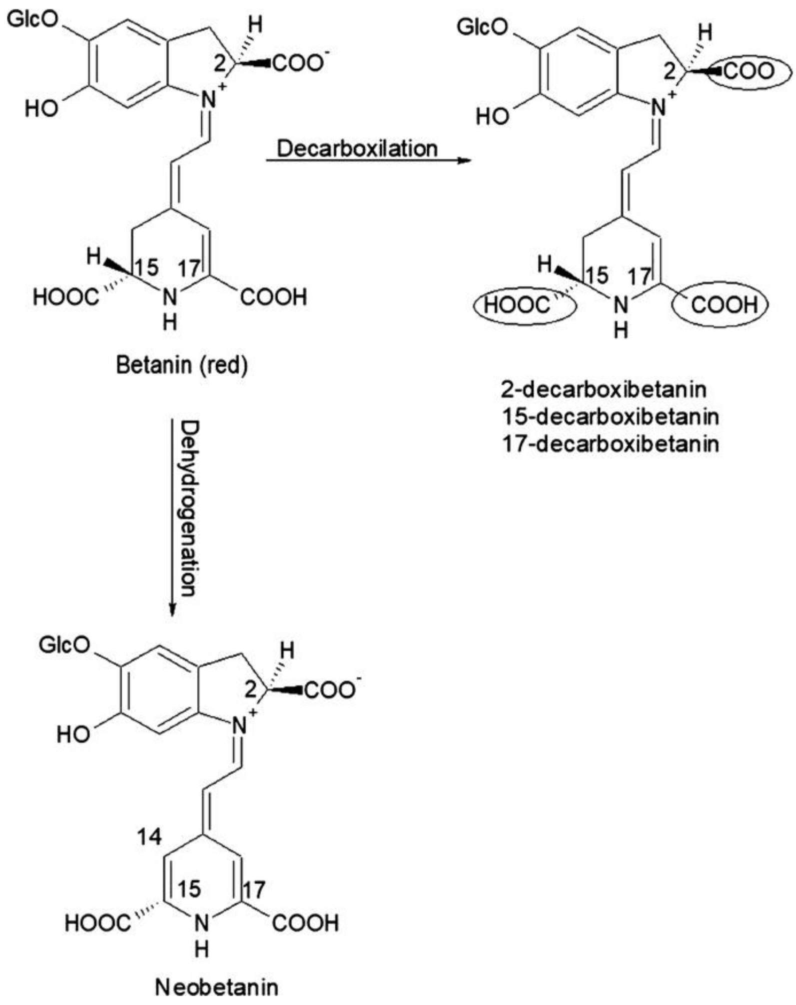

Figure 11. The degredation of betanin from beetroot that was obtained from IR [20].

Figure 12 proved that high temperature affects the stability of betanin by changing color. The red color of the solution started fading away after 1h (Fig. 12 (A)), leaving the sample longer in the high temperature water bath letting the color changed very clearly from red to brown yellowish color (Fig. 12 (B)). Whereas the flask that was left at RT did not show a clear change of its color.

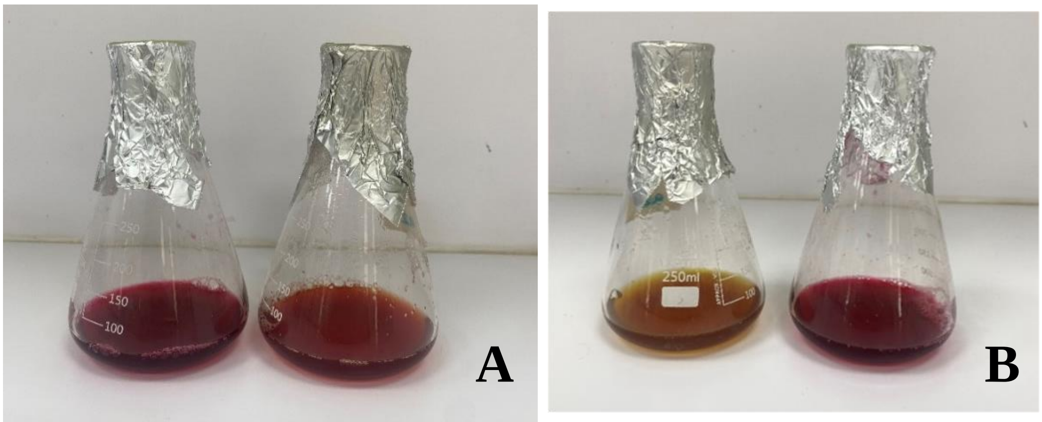

Figure 12. The samples after incubation for 1 h (A) the right flask was incubated in (75°C) water bath and the left one at RT. (B) the flask on the left was incubated in (75°C) water bath whereas the one on the right at RT.

4.4. Photostability of betanin

The procedure we followed to measure the Bc by studying the effect of different temperature was repeated again but this time with covering the flasks to avoid the light form reaching our samples. The purpose was to study the sensitivity and stability of the betanin in our sample when the light is prevented.

Figure 13 shows that the concentrations decreased with time in both temperature 75°C and RT. Same observation for the unexpected increase after the first hour of incubation for the sample that left at RT (Fig. 13 (B)); however, there was a significan decrase in the concentartion recorded. The comparison between figure 10 and 13 proved that the light does affect the stbility of the betanin and increased the degrading process of it. The betanin concentration at 75°C decreases about 2 fold when it was covered compared to 2.5 fold when it was not. Whereas the decreasing fold was about 1.5 comparing to 1.7 in covered and not covered samples, respectivly. This results agrees with published results that proved the ability of light to influence the satbility of betanin [7].

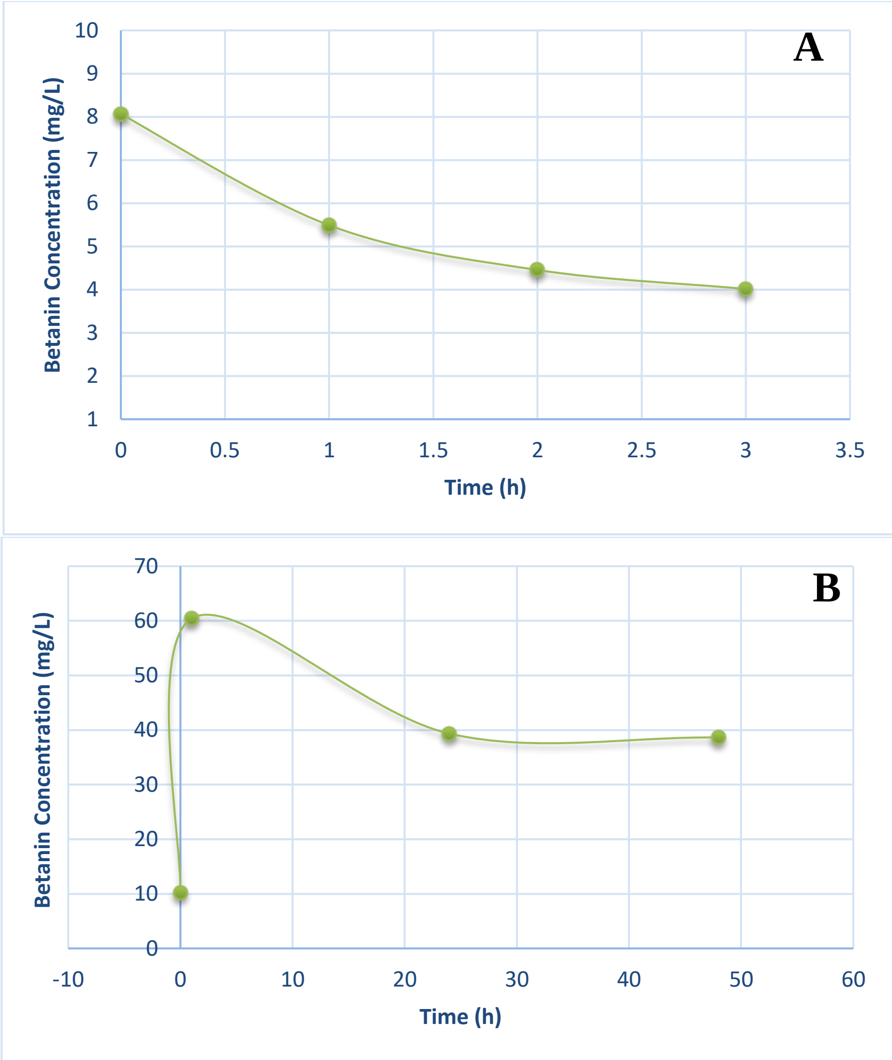

Figure 13. Bc was measured at a different time to study photostability. A) at a high temperature (75°C). B) at RT.

Chapter5

Conclusion & Recommendations

5.1. Conclusion

Beet root is a rich source of betalains and its derivatives. There are many ways to extract betanin from beet root. The way that has been used in this study was spectrophotometric method. The maximum visible absorption spectra were found in the range between 530 – 550 nm. The concentration for betanin was measured in different solid to liquid ratio and different DW to ethanol ratio. The highest content of betanin was found in 1:5 solid/liquid ratio and 1:1 DW/ethanol ratio.

The effect of temperature and light on the stability and the sensitivity of betanin compound was tested. The betanin structure is affected by the high temperature and light by increasing the rate of degradation. At 75°C the Bc decreased rapidly comparing to RT. Covering the extracts from light decreased the rate of the degradation at 75°C and RT.

5.2. Recommendations

For future work few recommendations can be followed:

1- Other extraction methods can be tested such as microwave, ultrasonic, or chromatography methods.

2- Other conditions and factors for the extraction can also be investigated such as changing the pH, changing the solvent mixture, adding metals, and oxygen.

3- Studying the antioxidant, anti-inflammatory, antimicrobial, and antiviral activities of betalains.

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