Study by Absorption and Emission Spectrophotometry of the Efficiency of the Binary Mixture (Ethanol-Water) on the Extraction of Betanin from Red Beetroot

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Research Article

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Abstract

In this paper, we intend to present a method of extraction and analysis of betanin from Algerian red beetroot, and to know the efficiency of the binary mixture (Ethanol-Water) on the extraction. This dye is a natural pigment with high antioxidant power and widely used as a natural dye. In addition, the high bioavailability of this pigment reinforces its potential for future therapeutic applications. However, the betanin content of commercially available beet extracts never exceeds 1.2% and this molecule does not exist marketed in a more purified form, it is therefore necessary to have efficient tools for the enrichment of betanin extracts. The experimental results made in our laboratory, confirm the effectiveness of the binary mixture (Ethanol-Water) on the extraction of betanin from Algerian beetroot.

Introduction

Vegetable beet (*Beta vulgaris* L.) has the notable scientific interest, because it is a rich source of nitrate (NO_3), a compound with advantageous cardiovascular health effects through the endogen production of nitric oxide (NO)[1,2].

Beetroot is the chief source of betalains[4-12] which is a water-soluble nitrogen pigment with heterocyclic ring, which can be further subdivided into two classes depending on chemical structure: betaxanthins comprising indicaxanthin; vulgaxanthin I and II, accountable for orange-yellow coloring; and betacyanins, such as betanin whish is a glucose heteroside (betanidine 5-O-glucose), and its aglycone is betanidine (Figure 1, 2), isobetanin, neobetanin, and prebetanin, accountable for red-violet coloring[4,5]. It is the best source for extracting the red color. Mainly using beetroot has several characteristics, including, for example the solubility in water, the stability over a pH range of 3-7, and it often used to color low-acid foods, and exist primarily in fruits and flowers, including a variety of fluorescents[6-20].

However, Betanin is the main component of the red dye extracted from beets which accounts for approximately 75-95% of all coloring matter found in beets[4-10]. It is available in the root beetroot, which is used as a coloring additive in foods[20-27]. It gives foods a desirable red color and has multiple uses in foods. In addition, betanin exists as an internal salt as a zwitterion in beet vacuoles. It is an anthocyanin which is a water soluble dye and gives colors ranging from purple and blue, to most shades of red. The color of betanin is pH dependent[9-15], at acidic pH (pH 4-5), it is red in color and gradually turns purple-red as the pH decreases (Figure 3). At alkaline pH (pH 11-12) betanin hydrolyzes and becomes yellow-brown in color (Figure 4). It is poorly soluble in oil (lipophilic)[5-14] and is soluble in water and ethanol (hydrophilic)[5-14]. Recently, medical researchers have discovered that betanin has bioactive properties such as an antioxidant, antimicrobial and anticancer [4,28,29].

Generally, the principal method used to isolate red food coloring is solid-liquid extraction, a process commonly used in the food industry. Being a water soluble pigment, betanin can be extracted from red

beets using any of the following solvents: acidified water, water-alcohol mixture or acidified alcoholic solution[16]. For example, Neagu and Barbu[17] studied the betanin extraction from beetroot using different solvent systems by solid-liquid extraction technique. These results revealed that the highest betanin content of around 20 mg/g of beet was obtained with the use of a weak acid solution (using 0,5% citric acid + 0,1 % ascorbic acid). In addition, they extracted the considerable amount of betanins using added solutions of ascorbic acid.

Analytical Extraction And Characterization

The separation method is physical, where ethanol and water were used as extraction solvents at different percentages in order to have a very good extraction yield and to observe the degree of selectivity of these solvents with respect to others previously used[27]. Commercially available Algerian beet has been used as extraction material. This plant material was raped and then filtered to obtain a juice from the beetroot. The extraction was carried out at 25°C with a time of 3 minutes. Before performing the spectrophotometric analyzes, several dilutions were prepared to obtain high resolution spectra. The dilution factor is around ¼.

The following solvents were used in the first series of experiments:

- Solution (20% Distilled water - 80% Ethanol)

- Solution (20% Ethanol - 80% Distilled water)

- Solution (40% Distilled water - 60% Ethanol)

- Solution (40% Ethanol - 60% Distilled water)

The used analysis methods are UV-Vis absorption spectrophotometry and emission spectrophotometry, the aim of which is to know the maximum wavelength absorbed or emitted by betanin extracted from beetroot. In addition, the amount of betanin extracted from 40 g of beet is calculated using the equation below (Eq.1).

$$m_i = \frac{A_i . F_d . M}{\varepsilon . l} . \frac{V_e}{1000 . m_s} \qquad \text{(Eq.1)}$$

Where:

m_i: quantity of betanin extracted per 40g of red beet used,

Ai: Sample absorption,

M: average molecular mass (550 $g.mol^{-1}$ for betanin),

ε: molar extinction coefficient (1120 $L.cm^{-1}. mole^{-1}$ for betanin) [21],

V_e: Volume of extract in ml (after extraction),

m_s: mass of red beet used for extraction,

F_d: Dilution factor (1/4).

Experimental Results And Discussion

The experimental results are expecting for the efficient extraction of betanin from beets. The UV-Vis absorption spectra of red beet extracts at different percentages of the solvents used are visible in the figure below (Figure 5). The shapes of the spectra show two peak centers (shoulder) at the wavelengths shown in the table below (Table 1).

From the results that we found (Table 1), we notice that betanin has an absorption maximum varying from 480nm to 540nm (Figure 5) and indeed our found values confirm the data of the literature[16,17]. As a result, when the binary mixture (Ethanol-Water) is used to extract betnin from red beet with a high percentage of water (80%), we've found an absorption band at 535nm and that it is the same value found when using a mixture (Ethanol-Water) with a low percentage of water (20%). For the mixture (Ethanol-Water) content 40% of water and 60% of ethanol, the maximum absorption wavelength appears at 539nm, however, for 60% water and 40% ethanol, the maximum absorption wavelength appears at 537nm. According to researches results[16,17], they showed that the maximum absorption limit of betanin occurs at a wavelength of 538nm at pH 5. In addition, they concluded that the aqueous extracts mainly contain betanidine and that the ethanolic extract mainly contains betanin, where a strong absorption band is observed at about 530nm in the visible range for beetroot juice which has been attributed to the betanin pigment.

In addition, an absorption band is observed at 532nm in the visible region using ethanol as a solvent. In addition, an absorption band at approximately 542nm with the intensity of absorption maxima for aqueous extracts is approximately equal, greater than the ethanolic extract. Thus, the researchers concluded that for the aqueous extract the second band had an absorption maximum at 515nm and for the aqueous extract at 509nm. These bands are only seen in the highly concentrated extract, and it disappears when the solution is diluted. Indeed, our analysis results by UV-Visible absorption spectrophotometry confirm their results.

For the absorbance given in Table 1, when using a mixture (Ethanol-Water) with a high percentage of water, the absorbance value exceeds 2 $moles^{-1}.cm^{-1}$; this explains why betanin is soluble in a mixture (ethanol-water) with a high percentage of water (80% water-20% ethanol). It is also noted that the quantities extracted from beet betanin are variable depending on the percentages of solvents used. The solvents (Ethanol-Water) used with a high percentage of water can extract a large amount of betanin from beetroot, i.e. approximately $3.42.10^{-4}$g/ml.

On the other hand, the UV-Vis emission spectra of beet extracts with the same percentages of solvents used are visible in Figure 6. It is known that betanin absorbs at 537 nm and emits at a wavelength longer than that absorbed. In order to know the value of the wavelength in which it will be emitted, we have chosen an emission range varying from 480 nm to 900nm, so that the value of the wavelength necessary to make this molecule excited must be less than 537nm. From the emission spectra obtained, it is observed that when a mixture (Ethanol-Water) with a small percentage of ethanol is used, the emission peak of this molecule appears at 537nm which is the same absorbed value. Also, when using a mixture (Ethanol-Water) with a high percentage of ethanol, this peak disappears, where it appears at 800nm. Therefore, we can say that the maximum betanin emission limit occurs at a wavelength of 800nm when using a mixture (Ethanol-Water) with high percentages of ethanol and low in water.

TABLE 1 Values of % in Ethanol /% in water ($X_{Ethanol}$, X_{water}), volume of the extract in betanin (Ve), mass of solid beetroot ($M_{Beetroot}$), calculated mass of betanin ($M_{Betanin}$), wavelengths maximum absorbed ($\lambda_{max1,2}$) and maximum absorbances ($A_{max1,2}$)

$X_{Ethanol}$	80%	60%	40%	20%
X_{water}	20%	40%	60%	80%
V_e(ml)	40,8	31,2	31,2	40,8
$M_{Beetroot}$(g)	40	40	40	40
$\lambda_{max1Absobed}$(nm)	478,5	481,5	480,5	480,5
A_{max1}	1,502	1,070	2,787	2,734
$\lambda_{max2Absorbed}$(nm)	535	539	537	535
A_{max2}	1,395	1,279	2,872	2,548
$M_{Betanin}$(g/ml)	$1,88.10^{-4}$	$1,2.10^{-4}$	$2,75.10^{-4}$	$3,42.10^{-4}$

Conclusion

The method of extracting betanin from Algerian beetroot, where ethanol and water are used as high water percentage extraction solvents, gives a good extraction yield. UV-Visible absorption spectrophotometry of the beet extract confirmed that betanin has an absorption maximum varying from 480nm to 540nm. The analyses by UV-Visible emission spectrophotometry of the beet extract are showed that the maximum betanin emission limit occurs at a wavelength of 800nm where ethanol and water are used as extraction solvents at high percentages of ethanol and low in water.

Declarations

Competing interests:

The authors declare no competing interests.

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Figures

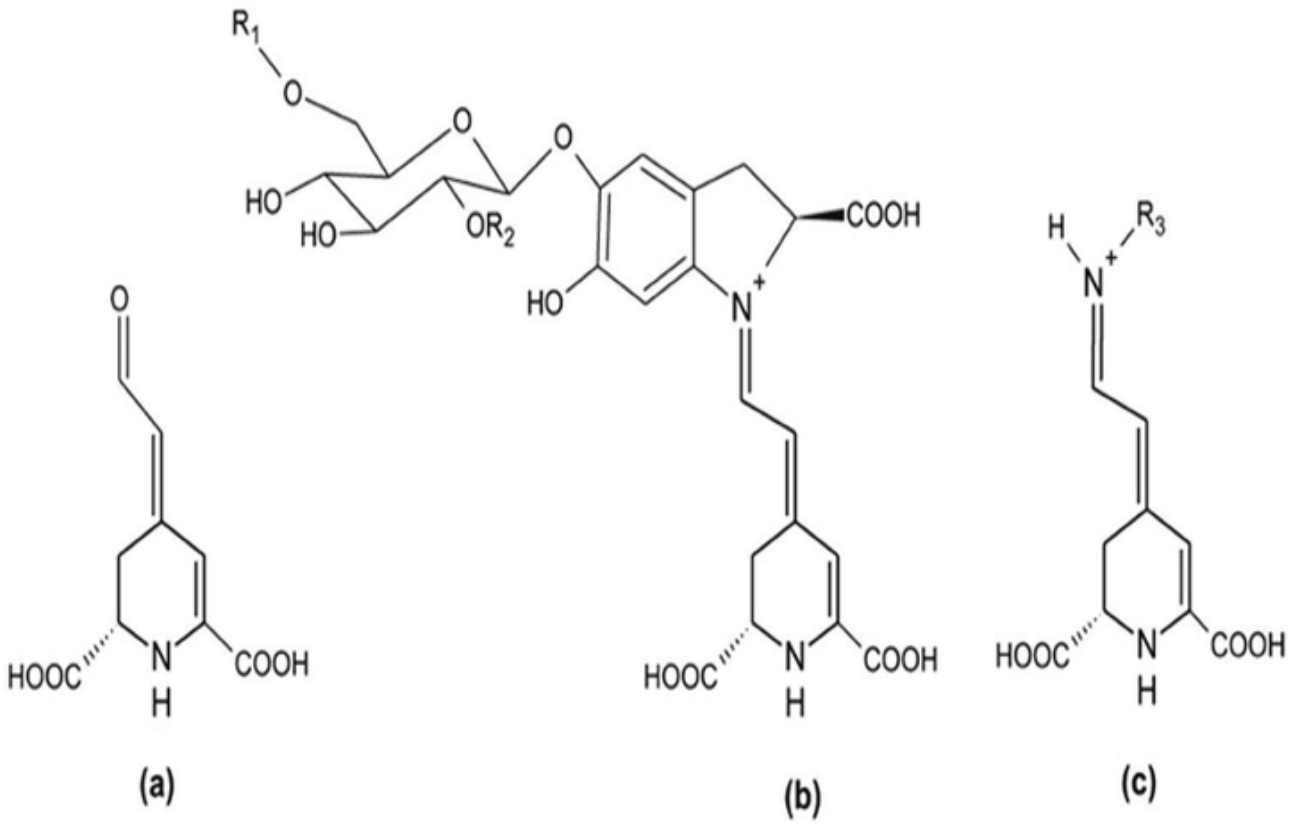

Figure 1

Betalamic acid (a), betacyanins (b) and betaxanthins (c)

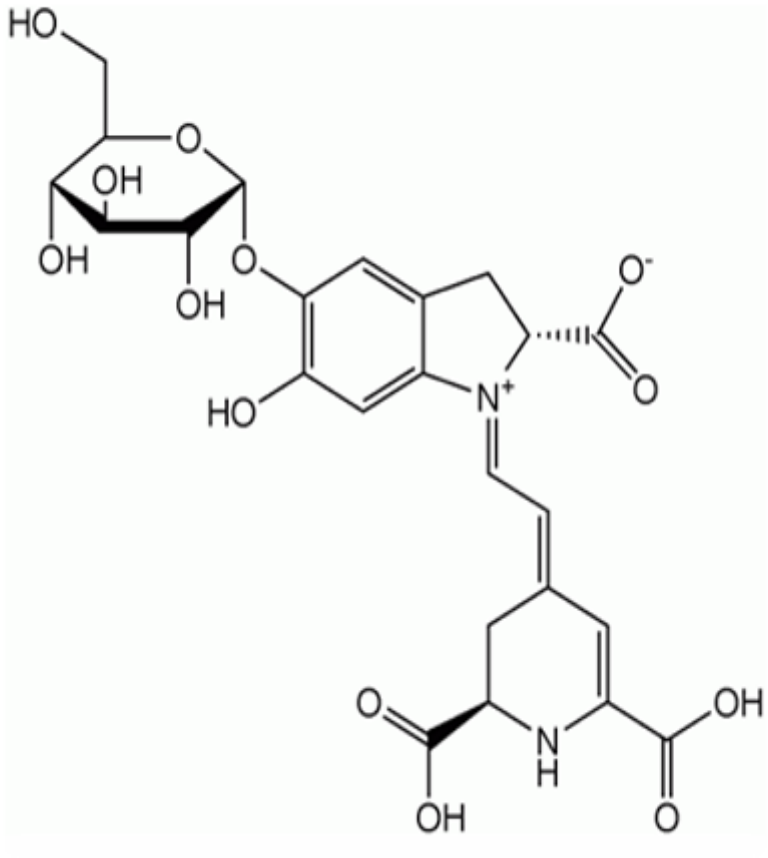

Figure 2

Structure of Betanin

Figure 3

Betanin powder of purple-red color

Figure 4

Betanin powder of yellow-brown color

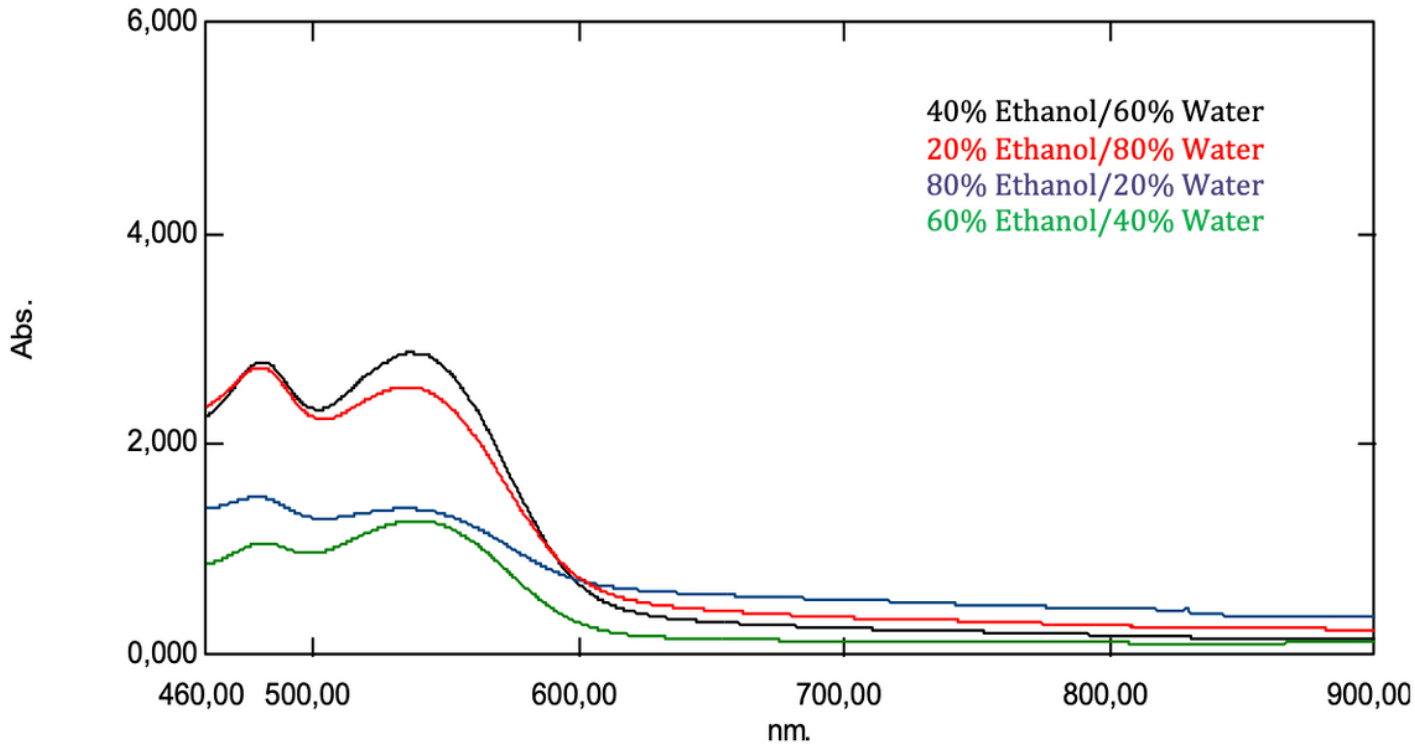

Figure 5

Uv-Visible absorption spectrum Beetroot extracts (% Ethanol /% Water)

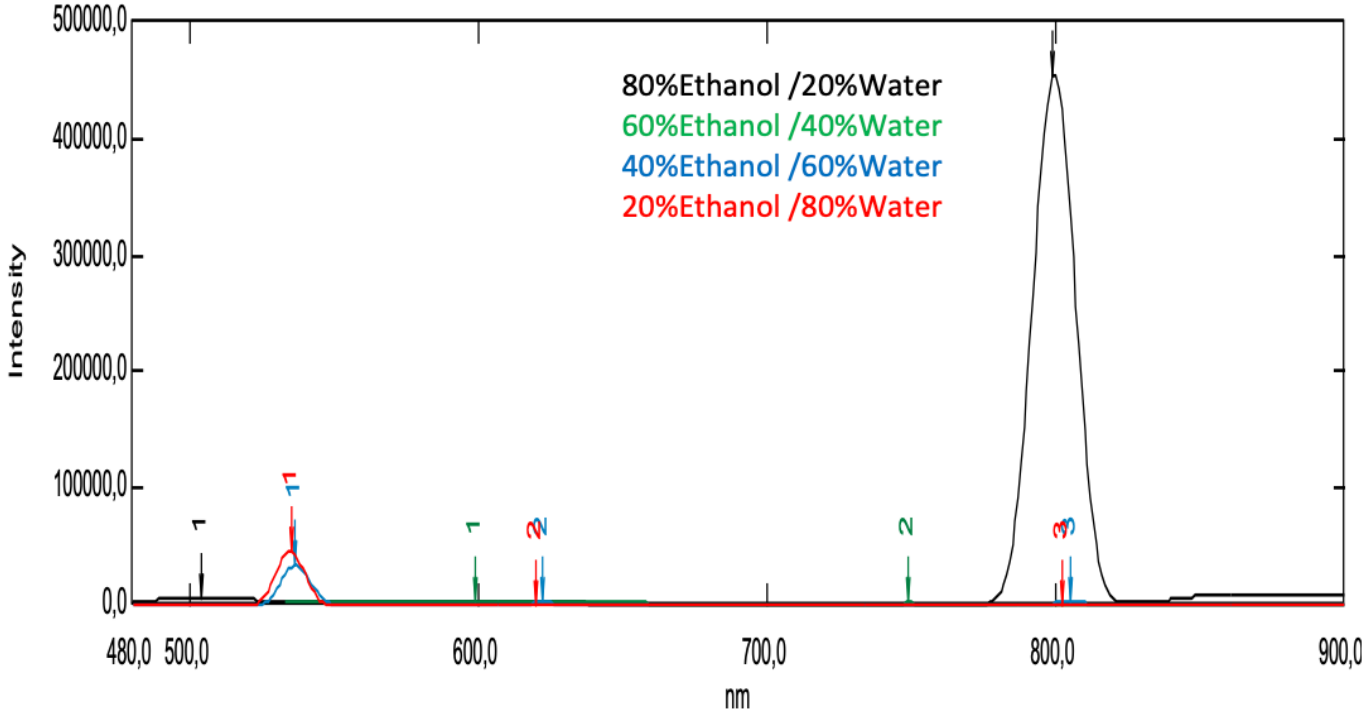

Figure 6

Uv-Visible emission spectrum Beetroot extracts (% Ethanol /% Water)