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Estimation of Some Vitamins in Beetroot Juice and Their Relationship to Cancer

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ABSTRACT

Background: The beetroot plant was purchased from the markets of Al-Najaf Governorate. The plant was washed with running water and the peels were removed from it, then cut into thin slices, dried away from sunlight and ground using an electric grinder. *Beta vulgaris* L. is known as beetroot or garden beet. It is classified as a species of the family Chenopodiaceae and it is a biennial herbaceous crop that grows all over the world for its edible roots and leaves. Beetroot varieties may be of yellow color up to deep red, but it is the red type that has wide consumption in the world. The multicolored look and good nutritional value attracted researchers interested in new food products . Betalains are among antioxidants found more specifically in red beet because of their anti-cancer activity.

Methods: Fine powder samples (5 mg) were extracted in 10 mL of methanol or distilled water and allowed to stand for 24 hours so that maximum solubilization of bioactive compounds could be achieved. The extract was then filtered to remove solid residues and the filtrates obtained were kept at 4°C for further use. All analyses were thereafter carried out in the laboratory of the Ministry of Science and Technology, Department of Environment and Water under controlled conditions to maintain sample integrity and accuracy of results. HPLC model It was utilized to analyses add detection of vitamins.

Result: Antioxidant activity was verified by DPPH assay where reduction of radicals appears as lower absorbance at 517 nm due to antioxidants. Phytochemical screening of methanolic extracts indicated a higher amount of bioactive compounds amongst which were total phenolics (120.5 mg/g DW), anthocyanins (60.55 mg/g DW), flavonoids (1.62 mg/g DW) and carotenoids (2.44 mg/100 g DW). Further quantification of vitamins by HPLC analysis revealed that in the methanolic extract, vitamin A was found to be 150.2 mg/100 g DW, vitamin E-8.6 mg/100 g DW and vitamin C-65.9 mg/100 g DW with the highest amount for vitamin A.

Key words: Anticancer activity, *Beta vulgaris*, Biological effects, DPPH, HPLC, Vitamins.

INTRODUCTION

Herbs have been very important in the lives of humans since time immemorial. They were used as means to keep good physical, psychological and spiritual health by many societies around the world. Some herbs may have one or more parts that develop bioactive compounds with real medicinal uses in practice-either applied directly as remedies or used as precursors in modern drug development (Davis and Choisy, 2024).

Beta vulgaris L. is known as beetroot or garden beet. It is classified as a species of the family Chenopodiaceae and it is a biennial herbaceous crop that grows all over the world for its edible roots and leaves (Kugler *et al.,* 2007). Beetroot varieties may be of yellow color up to deep red, but it is the red type that has wide consumption in the world (El-Beltagi *et al.,* 2018). The multicolored look and good nutritional value attracted researchers interested in new food products (Chaudhari and Nikam, 2015). Betalains are among antioxidants found more specifically in red beet because of their anti-cancer activity besides accompanying nutrients, as well as offering therapy; indeed, beets are among the top ten antioxidant-containing vegetables worldwide wherein these components act as free radical scavengers protecting protein and other cell structures from oxidative damage (Ibraheem *et al.,* 2015).

Beet leaves contain a great amount of antioxidants and vitamins, making them a very healthy food choice and at the same time an alternative to spinach. Betalain pigments from red beets have large commercial and pharmaceutical importance, such as natural colorants in food, cosmetics, pharmaceuticals and even art materials (Sainath *et al.,* 2016). Besides pigments, beets also include many bioactive components-phenolics, carotenoids betalains and essential nutrients that offer tremendous health advantages with low calorie intake contributing small amounts of dietary energy (Gamage *et al.,* 2016; Singh and Hathan, 2014).

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Biological effects of *Beta vulgaris*

1. Biological activity

Beta vulgaris has long been known for its positive health effects, mainly as a stimulator of the hemopoietic system in blood formation and immune system activity. It is a protective

agent of vital organs such as the kidney, liver and gastrointestinal tract against the insulting effects of toxic compounds and oxidative stress. Also, B. vulgaris shows mineralizing action helpful in the teaming up of tissue and bone health plus antiseptic and choleretic effect helping in microbial defense as well as in bile secretion. It further takes part in the strengthening of the gastric mucosa hence its role in the teaming up of the integrity of the digestive system. Red beetroot also exhibits a wide spectrum of pharmacological properties, including vasodilatory, cardioprotective, antiatherogenic, antithrombotic, antiallergenic, anti-inflammatory, antimicrobial and antioxidant actions (Rehman *et al.,* 2020).

2. Anti-inflammatory activity

The red pigment belongs to the principal betalains of beetroot (*Beta vulgaris*) and exerts powerful anti- inflammatory activities mainly through the inhibition of cyclooxygenase (COX) enzymes in their reaction pathways that cataly the conversion of arachidonic acid to prostaglandins and other eicosanoids-the major chemical mediators of inflammation. The pathway will make it be able to express her activity by suppressing this pathway the actual betanin suppression pro-inflammatory molecule production, therefore tissue damage and inflammatory response would also be limited (Yadav *et al*., 2016).

3. Antioxidant activity

Red beetroot ranks among the top ten vegetables worldwide in terms of antioxidant content. The antioxidants it contains act as free radical scavengers, thereby protecting vital biomolecules such as proteins, DNA and lipoproteins from oxidative injury (Ahmad, 2013; Muteab and AL-Abedy, 2025). By limiting this oxidative stress, the bioactive compounds in red beets contribute to the prevention of several chronic conditions, including cancer, cardiovascular disorders, cataract formation, neurodegenerative diseases and stroke that are closely linked to damage of cellular macromolecules (Gawlik-Dziki *et al.*, 2020). Shown how betalains are incorporated into human red blood cells and how this protects the cells from oxidative hemolysis (Klewicka *et al.,* 2012).

Due to the substantial quantity of betalain and the presence of additional phenolic compounds such as “ferulic, vanillic, p-hydroxybenzoic, caffeic and catechuic acid,” the beet pulp demonstrated an exceptional antioxidant action (Ferreira *et al.*, 2015).

4. Anticancer activity

Previous studies probing the anticancer potential of the Beta genus were primarily based on Beta vulgaris. It is within this species that betalains have been recognized as the major bioactive compounds responsible for expressing anticancer activity. Disruption of metabolite exchange between tumor cells and their surrounding microenvironment was one of the first mechanisms elicited in explaining their antitumor effect. Batalains interfered with this metabolic communication purportedly by reducing the ability of tumor cells to infiltrate adjacent tissues; thus, it would place its growth as well as invasive potential under some limitation. The findings further demonstrated that betanin triggered cell apoptosis via the intrinsic pathway, involving the mitochondrial release of cytochrome C and activation of Poly (ADP-Ribose) Polymerase (PARP). (PARP) cleavage (Ninfali *et al.,* 2017).

Medical uses

Red beetroot has been described as possessing biological properties antioxidants, antimicrobial, anti- inflammatory, vasodilatory, cardioprotective, antiatherogenic, antithrombotic and antiallergenic actions (Baião *et al.,* 2017). Mainly beet juice contains antioxidants reducing system inflammation helping regulate pressure. It is also noted that beetroot juice possesses a higher content of nitrates and therefore recommendation for intake in athletes since it improves endurance and overall performance during activity (Da-Silva *et al.,* 2016). Studies have indicated dietary nitrates from beetroots are converted within thehuman organism into nitric oxide (NO) which then results in vasodilatation giving better blood flow lowering vascular resistance; thus probably lowering blood pressure (Munekata *et al.,* 2021). Though these findings are encouraging, existing evidence is inadequate to fully establish the role of beetroot juice in therapy as a reliable therapeutic intervention for chronic hypertension until large-scale clinical trials further reinforce it (Ingle *et al.,* 2017).

MATERIALS AND METHODS

Preparing the plant for study

The beetroot plant was purchased from the markets of Al-Najaf Governorate. The plant was washed with running water and the peels were removed from it, then cut into thin slices, dried away from sunlight and ground using an electric grinder.

Extraction method

The alcoholic fruit extract was prepared in the Pharmacognosy Laboratory / College of Pharmacy / Al-Kafeel University in March 2022, from the powder of plant fruit according to the (AL-Aridhee, 2018). Fine powder samples (5 mg) were extracted in 10 mL of methanol or distilled water and allowed to stand for 24 hours so that maximum solubilization of bioactive compounds could be achieved. The extract was then filtered to remove solid residues and the filtrates obtained were kept at 4°C for further use (Sumathy and Sumathy, 2011). All analyses were thereafter carried out in the laboratory of the Ministry of Science and Technology, Department of Environment and Water in Baghdad under controlled conditions to maintain sample integrity and accuracy of results.

Chemical constituents from Red beets methanol extract (El-Beltagi *et al.,* 2018):

1. Total polyphenol content

The total polyphenol content was estimated by Folin Ciocalteu method, The absorbance was assessed at 765 nm using a spectrophotometer Thermo Scientific HERYIOS.

2. Total flavonoid content

Flavonoid content estimation of the sample was carried out by the aluminum chloride colorimetric method, which is traditionally adopted for flavonoid determination. In this set-up, a reaction mixture with aluminum trichloride (AlCl*f*) forms a stable complex corresponding to flavonoid compounds available in the extract. The intensity of color developed was read at 510 nm using a UV-visible spectrophotometer. There exists direct proportionality between absorbance and the concentration of flavonoids available in the extract.

3. Anthocyanin concentration

The fresh tissues of *Beta vulgaris* were blended in methanol supplemented with 1% hydrochloric acid (v/v). The obtained extract was filtered and its absorbance values were measured at 530 nm and 657 nm using a spectrophotometer**.**

4. Carotenoid concentration

The carotenoid pigments from red beet samples were isolated employing a solvent mixture of hexane and acetone in equal volumes (1:1, v/v). The absorbance of the resulting extract was determined at 630 nm with the aid of a spectrophotometer.

Antioxidant extract

Assessment of antioxidant strength

The potential for antioxidants was found by the DPPH test using known methods. A stock mix of DPPH was made by mixing 0.04 g of the compound in 100 mL of methanol, giving a final amount of 400 µg/mL (Suganyadevi *et al.,* 2010). For the preparation of the reference standard (Vitamin C) and test samples, dissolve 0.5 g of vitamin C in 100 mL methanol-distilled water mixture to achieve a concentration of 5000 ppm. From this stock, prepare serial dilutions to obtain concentrations of 30, 60, 120, 250 and 500 ppm for both standard and sample solutions.

Mix well and let it sit for 30 minutes at 20 C. Absorbance has been assessed at 517 nm using UV-VIS spectro-photometer (Shimadzu). The IC_{50} is defined as the quantity of sampe needed to scavenge 50% of the DPPH free radicals and was measured using a log-dose inhibition curve. Low absorbance means high activity for free radical quenching.

You can figure out how much DPPH radical scavenging activity is happening with this formula:

$$\%DPPH = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Chromatographic methods

1. Identification of vitamins by HPLC

HPLC model SYKAM (Germany) It was utilized to analyses add detection of vitamins, in the Ministry of Science and Technology/ Baghdad. Chromatographic Conditions: The isocratic conditions were used with a mobile phase composed of acetonitrile and distilled water, 75:25 (v/v). The flow rate was kept at 0.7 mL/min for the entire run. A reverse-phase C18-ODS analytical column of dimensions 25 cm × 4.6 mm, with a particle size of 5 µm has been used to generate very good resolution. UV detection at 280 nm was employed since most aromatic compounds as well as phenolic structures absorb strongly at this wavelength. System equilibration before an injection ensured baseline stability and the reproducibility of results obtained (Fig 1).

2. Determination of water - fat soluble vitamins

0.1% solution of butylhydroxytoluene in methanol (solution no. 2). To analyze fat-soluble vitamins, 0.05-0.1 g of the sample was mixed with 5 mL of solution no. 2, placed in a sealed container and kept in the dark for 2 hours. The mixture was then agitated at room temperature for 20 minutes. Prior to chromatographic analysis, the samples were centrifuged and the resulting extracts were injected into the column within 1 hour (Karaźniewicz-Łada and Główka, 2016).

Concentration of sample was calculated as following :

$$\text{Concentration of a sample} = \frac{\text{The Area of the sample}}{\text{Area of standard} \times \text{con. of standard} \times \text{dilution factor}}$$

$$\text{Con. of sample} = \frac{13261.644}{678.554^{*}\ 25^{*}10 = 488\ \text{pp m}}$$

RESULTS AND DISCUSSION

Red beet (*Beta vulgaris* L.) is a root vegetable characterized by its low fat content. It is, however, a good source of carbohydrates, starch, soluble dietary fibers and proteins, making it a food of moderate caloric density (Table 1).

Antioxidant activity of *Beta vulgaris*

DPPH Scavenging activity

The sample was compared with one of the types of antioxidants, vitamin C, where several concentrations of the vitamin and the sample were prepared and according to the method, the ability of each concentration to remove free radicals was known, The DPPH antioxidant activity is mostly related to its ability to donate hydrogen atoms. DPPH is a stable free radical and antioxidants can reduce it either by donation of electrons or hydrogen atoms leading to the formation of reduced non-radical diamagnetic molecules. The DPPH radical scavenging ability of the samples was evaluated by measuring the reduction in absorbance at 517 nm. This reaction is also evident by a visible color change from deep purple to yellow. The antioxidant activity showed a concentration-dependent effect, with radical scavenging activity increasing progressively at extract concentrations of 30, 60, 120, 250 and 500 µg/mL. The methanolic extract of red beet exhibited an IC_{50} value of 67.812 µg/mL. The IC_{50} represents the concentration of the extract necessary to suppress 50% of free radical activity. This parameter is commonly employed as an indicator of antioxidant potential (El-Beltagi *et al.*, 2018). Notably, lower

IC_{50} values reflect stronger free radical scavenging capacity. (Table 2).

Chemical constituents of red beet beetroot

As illustrated in (Table 3), the chemical constituents of methanolic extract of red beet contain total phenolic (120.5 mg/g DW), total athocyanin (60.55 mg/g DW), total flavonoids (1.62 mg/g DW) and carotenoids (2.44 mg/100 g DW). These findings are identical to previous studies [40-42], who reported that the major components of red beets extract are polyphenols, flavonoids, In addition, to carotenoids (Table 3).

The identification of vitamins by high performance liquid chromatogram (HPLC)

The HPLC analysis of Antioxidant (Vitamins**)** standard of *Beta vulgaris* beetroot successfully content Vitamins 488 ppm is shown in Table (4).
The following charts were obtained.

HPLC of soluble vitamins

Table 5 shows that the red beet methanolic extract is high in vitamins with vitamin A at 150.2 mg/100 g DW, vitamin E at 8.6 mg/100 g DW and vitamin C at 65.9 mg/100 g DW. This finding tallies with the study by Abdullah *et al.* (2023) who also found high levels of vitamins in red beets, particularly vitamin A at 150.2 mg/100 g. The functions performed by vitamin A in human health include growth and tissue maintenance, hormone production, synthesis of neurotransmitters and proper immune function (Gaurav *et al.,* 2016). Vitamin C has basic antioxidant properties whereby it nullifies any dangerous effects caused by reactive oxygen species (ROS). It prevents macromolecules-lipids, proteins and DNA from oxidative damage; hence playing a protective role against chronic conditions such as cancer, cardiovascular disease, disease, or neurodegenerative disorders. (Naidu, 2003).

Quantitative analysis

The retention time of the standard substance was compared with the time closest to appearing in the Sample, so the vitamins were diagnosed in the Sample:

1. Vitamin A

The results shown in Table (6) content *Beta vulgaris* of Vitamin A with retention time 5.95 min which is 10 ppm.

2. Vitamin E

The results shown in Table (7) content *Beta vulgaris* of Vitamin E with retention time 3.15 min which is 10 ppm.

3. Vitamin C

The results shown in Table (8) content *Beta vulgaris* of Vitamin C with retention time 4.18 min which is 10 ppm.

This study is in line with a trend that aims to efficiently discover different antioxidants and vitamins in varying amounts found in red beetroot and determine its preventative role in creating pharmaceutical medications with fewer

Table 1: Scientific classification of *Beta vulgaris* L. (Tomer *et al.,* 2020).

Kingdom	Plantae
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Caryophyllidae
Order	Caryophyllales
Family	Chenopodiaceae
Genus	*Beta*
Species	*Vulgaris*
Subspecies	*Beta vulgaris* L. subsp. *vulgaris*

Table 2: Antioxidant activity of *Beta vulgaris* by DPPH method.

AA%	30	60	120	250	500	IC_{50} (µg/ml)
Vit C	15.90	25.69	40.25	66.25	78.91	45.4
Sample	30.25	52.61	71.56	88.98	95.66	67.812



Fig 1: Cromatography HPLC.

Table 3: Quantitative phytochemical analysis of *Beta vulgaris.*

Ingredients	Levels in methanolic extract
Total phenolic (mg Gallic acid /g DW)	120.5±0.10
Total athocyanin (µg/100g FW)	60.55±0.020
Total flavonoid (mg Quercetin /g DW)	1.62±0.07
Carotenoids (mg/100g FW)	2.44±0.037

Table 4: HPLC of standard vitamins.

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05[min]
1	2.28	4320.56	252.15	15.26	15.24	0.10
2	3.19	11542.05	360.85	21.59	21.22	0.15
3	4.15	12653.99	613.65	27.25	27.65	0.25
4	5.92	8856.08	450.55	19.59	19.05	0.20
5	7.49	5996.25	313.65	14.66	14.20	0.15
	Total	43368.15	1990.85	100.00	100.00	

Table 5: Water soluble vitamins contents of *Beta vulgaris.*

VIT C (PPM)	VIT E (PPM)	VIT A (PPM)
65.9	8.6	150.2

Table 6: HPLC of vitamin A.

Retention Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05[min]
5.95	1477.08	688.98	100.00	100.00	0.25
Total	1477.08	688.98	100.00	100.00	

Table 7: HPLC of Vtamin E.

Retention time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]
3.15	2055.49	921.55	100.00	100.00	0.25
Total	2055.49	921.55	100.00	100.00	

Table 8: HPLC of Vitamin C.

Retention time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]
4.18	1203.08	530.25	100.00	100.00	0.25
Total	1203.08	530.25	100.00	100.00	

adverse effects (Jedlicka and Klimes, 2005). The current study's *in vitro* tests offer strong proof that red beet, an inedible waste product, has strong antibacterial, antioxidant and anticancer properties, suggesting that it can be utilized as a functional ingredient (Vasconcellos *et al.*, 2016).

CONCLUSION

Processed beetroot shows great stability and significant antioxidant potential. Phytochemical screening has verified that all extracts from *Beta vulgaris* contain bioactive compounds as phenolics, flavonoids, anthocyanins and carotenoids abundantly. Beta vulgaris is consumed largely in the world both fresh and cooked due not only to its palatability but also high nutritional and therapeutic values. This is considered a functional food that promotes health, the prevention of illnesses and even the treatment of many conditions. Beetroot helps prevent chronic diseases such as heart diseases, diabetes, or even cancer. Beta vulgaris has been further proved by this study to carry a relatively high amount of different essential vitamins at different concentrations.

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Disclaimers

The views and conclusions expressed in this research are solely those of the author and do not necessarily reflect the

official policies or positions of the affiliated institution, sponsor, or publisher. The authors bear full responsibility for the content of this work.

Informed consent

Informed consent is not required for this research study, as it was carried out on beetroot plant and does not include any human participants.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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