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International Journal of Current Research
Vol. 5, Issue, 12, pp. 3723-3728, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

QUANTITATIVE PHYTOCHEMICAL, PROXIMATE/NUTRITIVE COMPOSITION ANALYSIS OF *Beta vulgaris* LINNAEUS (CHENOPODIACEAE)

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ARTICLE INFO

Article History:
Received 11th September, 2013
Received in revised form
02nd October, 2013
Accepted 21st November, 2013
Published online 02nd December, 2013

Key words:
Beta vulgaris,
Quantitative phytochemical analysis,
Proximate composition,
Elemental analysis,
Vitamins.

ABSTRACT

The quantitative phytochemical analysis, proximate composition and level of some nutrients of *Beta vulgaris* (Beet root) were studied using standard analytical method. Result of the quantitative phytochemical analysis indicates the presence of alkaloids (128.889), steroids (16.4), glycosides (0.652), flavonoids (6.417), terpeniods (115.5), saponins (3.789), and acidity level (5.227) all in mg/100g. Proximate composition analysis of *Beta vulgaris* (Beet root) indicates that it contains 1.35, 0.3, 1.9, 2.56, 87.4 and 1.4 % of protein, fats and oils, dietary fibre, total fibre, moisture, and ash value respectively, β-carotene (11.64 mg/100g) and energy (42 kcal). The elemental analysis also indicates the presence of the following minerals: iron, magnesium, copper, sodium, potassium, manganese, calcium and zinc in these ratio 0.76, 18.60, 0.08, 73.60, 31.20, 0.86, 13.80 and 0.29mg/100g respectively. Vitamins found were vitamin A (2.6µg/100g), vitamin K (3.2µg/100g), vitamin C, vitamin E, vitamin B3, vitamin B6, vitamin B2, vitamin B, panthotenic acid and cholesterol (4.36, 0.18, 0.35, 0.03, 90.053, 0.034, 0.151 and 0.04 mg/100g respaectively). This result reveals that the root contain appreciable amounts of nutrients that justifies its use in treatment of different ailments.

INTRODUCTION

Plants are primary source of medicines, fibre, food shelters and other items in everyday use by humans with roots, stems, leaves, flowers, fruits and seeds providing food for humans (Hemingway, 2004). Plants serve as an indispensable constituent of human diet supplying the body with minerals salts, vitamins and certain hormone precursors, in addition to protein and energy (Oyenuga & Fetuga, 1975). Seeds have nutritive and calorific values which make them necessary in diets (Odemelam, 2005). The plant part commonly eaten is the seeds which are either cooked or eaten raw. Okafor (1993) reported that some plant species were in the process of being lost. Recently, a total of thirty plant species producing edible fruits, seeds, leaves and bulbs in south-eastern Nigeria rainforest have been reported as endangered (Meregini, 2005). Many of these edible fruits, seeds, leaves and bulbs were collected from the wild and their habitats are currently threatened irrespective of their uses among man. There is paucity of literature on the proximate, phytochemical and nutrient compositions of these fruits/seeds, leaves and bulbs. There is need to understand the suitability for either food or fodder. A proper understanding of their proximate, phytochemical and nutrient composition will lower the over dependence of many communities and industries on few known arable crops for fruits, seeds, vegetable and bulbs. Also knowledge of their composition would enable one to know the better type of fruits, seeds leaves and bulbs to eat or feed to animals at any given time. *Beta vulgaris* (Chenopediceae) also known as garden beet or red beet in the US scientific literature and beetroot in Europe and many other countries around the world, is a herbaceous biennial or rarely perennial plant. It has a long history dating to the second millennium BC. The first cultivated forms were believed to have been domesticated in the Mediterranean, but were introduced to the Middle East, India and finally China by 850AD.

These were used as medicinal plants in Ancient Greece and medieval Europe. Their popularity declined in Europe following the introduction of spinach. *Beta vulgaris* is best known in its numerous cultivated varieties, the best known of which is the purple root vegetable known as the beetroot or garden beet. The "earthly" taste of some beet root cultivars comes from the presence of geosimin. Researchers have not yet answered whether it is produced by symbiotic soil microbes living in the plant (Lu *et al.*, 2003). Beetroot contains the bioactive agent betaine which supports healthy liver function (Vali *et al*., 2007). It was found that beet juice inhibits or enhances N-nitrosodimethylamine (NDMA) formation (Kapadia *et al*., 1996). In a study to determine the estimated Glycemic index of various foods, it was concluded that

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beetroot has a medium G.I of 64 (Babek *et al*., 2000). It is used in treatment of cardiovascular diseases (Olth *et al.,* 2005) and blood pressure (Ahulwalia, 2008). Proximate and nutrient analysis of medicinal plants, edible fruits and vegetables plays a crucial role in assessing their nutritional significance (Pandey *et al*., 2006). As various medicinal plant species are also consumed as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species ((Pandey *et al.*, 2006). This study therefore focuses on the phytochemical, proximate composition, mineral analysis of *Beta vulgaris* root with a view to assess the nutritional potential in relation to its ethnomedicinal uses.

Experimental

Collection and preparation of plant material

The root of *Beta vulgaris* were collected during the month of February, 2011 at Nsukka, Enugu State, Nigeria and authenticated by Mr. A Ozioko, A botanist with the International Centre for Ethnomedicine and Drug Development (InterCEDD) Nsukka. The voucher specimen (UNN/PCOG/010/409) was deposited in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. Dirty and sand were removed from the roots by rinsing in clean water. The roots were sun-dried for six days and pulverized using an electric blender into a fine powder of 60 mesh sieve size which was used for the various analyses.

Preparation of standard

Stock solutions of iron, potassium, calcium, sodium, and magnesium were prepared in accordance with standard methods (Association of Official Analytical Chemistry (AOAC, 1995).

Qualitative/quantitative phytochemical analysis

Alkaliods determination

The determination was as described by Harborne (1973). A portion (5 g) of sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoids determination

This was done following the method of Boham & Kocipai-Abyazan (1994). A 10 g of the sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Cyanogenetic glycoside determination

The extraction was according to Wang and Filled method as described by Onwuka (2005). A portion (5 g) of the sample was made into a paste and the paste was dissolved into 50 ml distilled water. The extract was filtered and the filtrate was used for cyanide determination. To 1 ml of the sample filtrate, 4 ml of alkaline picrate was added and absorbance was recorded at 500nm and cyanide content was extrapolated from a cyanide standard curve.

$$\text{Cyanide (mg/g)} = \frac{\text{Absorbance x CF x DF}}{\text{Sample weight}}$$

Where GF = gradient factor and DF = dilution factor

Saponins determination

The method employed was that of Obadoni and Ochuko (2001). A portion (20 g) of the sample was put into a conical flask and 100 ml of 20 % aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55 ^{0}C. The mixture was filtered and the residue re-extracted with 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 ^{o}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether was discarded. The purification process was repeated. 60 ml of n-butanol wa added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated on a water bath. After evapouration the samples were dried in the oven to a constant weight. The saponins content was calculated as percentage.

Tannins determination

Tannin determination was done by Van-Burden and Robinson (1981) method. A portion (500 mg) of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Phytic acid determination

Phytic acid was determined using the procedure described by Lucas and Markakas (1975). A portion (2 g) of the sample was weighed into 250 ml conical flask. 100 ml of 2 % concentrated hydrochloric acid was used to soak the sample for 3 h. The mixture was filtered and 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added to give proper acidity. 10 ml of 0.3 % ammonium thiocyanate solution was added to the solution as indicator and was titrated with standard iron chloride solution which contained 0.00195 g of iron per ml.

% Phytic acid = y x 1.19 x 100
Where y = titre value x 0.00195

Proximate Analysis

The proximate analysis (moisture, ash, carbohydrate, fats, protein, moisture, fibre, energy and β-Carotenes) of *Beta vulgaris* was determined by using AOAC (2005) methods. The

moisture and ash were determined using weight difference method. Carbohydrate was determined by difference method: the sum total of the moisture, fat, protein, and ash content of each part of the samples were subtracted from 100 as follows: Carbohydrate content = 100 – [protein (%) + fat (%) + moisture (%) + ash (%)]. The nitrogen value which is the precursor for protein of a substance was determined by Micro-Kjeldahl method. The nitrogen value was converted to protein by multiplying to a factor of 6.25. Crude fat was determined using oven-dried samples from moisture content determination. This was extracted with petroleum ether (boiling point (BP) 40 to 60°C) for 6 h with soxhlet extractor. After evaporation of ether, drying to constant weight, and cooling, the result was expressed in percentage. The sample calorific value was estimated (in kcal) by multiplying the percentage crude protein, crude lipid, and carbohydrate by the recommended factor (2.44, 8.37, and 3.57, respectively) used in vegetable analysis (Asibey-Berko & Tayie, 1999). Crude fibre content was determined using Gallenkhamp muffle furnace at 550°C and the result was expressed in percentage. The β-carotene was determined by taking the absorbance using a spectrophotometer (model 22UV/VIS) at a wavelength of 436nm.The concentration of β-carotene was calculated using Bear-Lamberts Law, which states that the absorbance (A) is proportional to the concentration(C) of the pigment, as represented by the equation:

$A \propto L$ (if concentration(C) is constant).
$A=ECL$; $C=A/EL$

Where:

C= concentration of carotene, A= absorbance,
E=extinction coefficient,
L= thickness of cuvettes (path length) =1cm,
E of β-carotene =1.25x104µg/l

Mineral Analysis

The sample was investigated for mineral composition (sodium, calcium, potassium magnesium, and iron) as outlined by Shah *et al.* (2009) for plant samples by using atomic absorption spectrophotometer (AAS), Bulk Scientific model AVG 210. A 2 g of the processed sample weighed was subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a Gallenkhamp muffle furnace. The resultant ash was dissolved in 5 ml of HNO3/HCl/H2O (1:2:3) and was heated gently on a hot plate until brown fumes disappeared. To the remaining material in the crucible, 5 ml of de-ionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through Whatman Grade No. 42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). A 10 cm long cell was used and the concentration of each element in the sample was calculated in the percentage of dry matter, that is, mg/100 g sample. Appropriate working standard solution was prepared for each mineral. The calibration curves were obtained for concentration versus absorbance.

Vitamins analysis

Vitamins were determined titrimetrically.

Statistical analysis

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULTS AND DISCUSSION

The result of phytochemical analysis (Table1) indicated that the root of B. vulgaris is rich in phytochemicals such as alkaloids, flavoniods, tannins, saponins, terpenoids, cyanogenetic glycosides, steroids and reducing sugars. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity (Sofowara, 1993). Phytochemical components are responsible for both pharmacological and toxic activities in plants (Ibrahim et al., 2000). They are used for therapeutic purposes to cure various diseases and to heal injuries (Okwu & Josiah, 2006). For instance, flavonoids have been shown to have antibacterial, anti-inflammatory, anti allergic, antiviral, antineoplastic (Alan & Miller, 1996; Mishra *et al*., 2008). Many of these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chelators (Nakayama *et al.*, 1993). Alkaloids contribute to plant species fitness of survival. They often have pharmacological effects and are used as medication and recreational drugs (Roger & Wink, 1998). They produce bitter taste that repels insects from feeding on plant leaves. The high alkaloid content could be contributory to the medicinal value of beetroot. The positive effect of glycosides and cyanogenetic glycosides are not common but their toxic effects include decreased heart rate, symphatetic activity and systematic vascular resistance (Siegler, 1998).

Table 1. Qualitative and quantitative phytochemical analysis of *Beta vulgaris*

Constituents	Value (mg/100g)
Reducing sugars	1458 ± 0.34
Tannins	6.055 ± 1.08
Saponnins	3.780 ± 0.55
Alkaloids	128.90 ± 0.40
Flavonoids	6.417 ± 0.22
Terpenoids	115.5 ± 1.20
Glycosides	0.652 ± 1.48
Steroids	16.4 ± 0.30
Acidity	5.22 ± 0.15

Valves are mean ± deviation of three replicate analyses

Cyanogenetic glycoside concentration in *B. vulgaris* parts studied appears very low. The presence of some of these anti-nutrients can be reduced by various processing techniques (Siegler, 1998). Saponins have reported to show tumor inhibiting activity on experimental animals (Akindahunsi & Salawu, 2005). The presence of saponins can control human cardiovascular disease and reduce blood cholesterol. Tannins may provide protection against microbial degradation of dietary proteins in the rumen (Aletor, 1993). Steriodal compounds are of importance in pharmacy because of their relationship with compounds used as sex hormones (Okwu, 2001). Phytic acid is present in beetroot and it has been reported that its chelating effect inhibits or even cure some cancers by depriving those cells of mineral (especially iron)

they need. The deprivation of essential mineral like iron would much like other serve as broad treatment for cancers (Hunell, 2003). Phytic acid can also be used as preservative and also food additive (Malleshi & Desikanchar, 1980). The results of the proximate and chemical compositions showing the moisture content, ash content, crude protein, crude lipid, crude fibre, carbohydrate, β-carotene and energy values is shown in Table 2. Moisture content was 87.4 % which is within the reported range (79 – 96 %) for root vegetables (Claudia, 1992).

Table 2. Proximate composition analysis of *Beta vulgaris*

Parameters	Value
Proteins	1.35 %
Fats and oils	0.3 %
Carbohydrates	6.99 %
Dietary fibre	1.9 %
Total fibre	2.56 %
Energy	42 Kcal/100g
Moisture	87.4 %
Ash	1.4 %
β-carotene	11.64 µg/100g

All vegetables have high water content. George (2003) reported that high moisture content is important in maintaining the protoplasmic content of the cells, but it makes the vegetables perishable and susceptible to spoilage by micro-organisms during storage. Beetroot is very low in calories (contain only 42 Kcal/100g), which is within the reported range of 10 – 85 Kcal for root vegetables (Claudia, 1992) and fat, but is very rich in dietary fibres, vitamins, and minerals. Vegetables have a low energy value. They generally provide between 10 Kcal and 50 Kcal (40-200 Kj) per 100g to obtain about 1000 Kcal, it would be necessary to eat about 2-3 kg. Their nutritional advantage is that they offer a high concentration of micronutrients for low contents of calories and fat. Virtually every national or international report on diet and health recommendations calls for an increase in fruit and vegetable consumption to replace high-energy foods (Smith and Eyzaguine, 2007). The fat content of 0.3 % showed that it can be considered as poor oil species and therefore cannot be used as a source oil for industrial or domestic purposes (Manish & Subhash, 2006).The carbohydrate content was low (6.99 %). Vegetables are composed chiefly of carbohydrates, mainly simple sugars and complex carbohydrates (starch and dietary fiber).

The content ranges from 1-2 % in the leaf and stem vegetables to 27% in root vegetables. Root vegetables have the highest carbohydrate content. Dietary fiber content ranges from 0.8 % in cucumber to 8.0 % in .carrot. It is known that carbohydrates contribute the greatest quota of energy required by man and animal (Oko & Onyekwere, 2010). The low fat and carbohydrate contents of the samples recorded in the present study supports the low energetic value observed in beetroot. High concentration of calcium (Ca), sodium (Na), potassium (K) and Magnesium (Mg) have been found in beetroot with Na having the highest concentration (Table 3). On the average, the increasing order of the macronutrients is Mg > Ca >K > Na. Minerals are known to play important metabolic and physiologic roles in living systems (Enechi & Odonwodo, 2003). The presence of minerals in supplementary diets is necessary to avoid metal deficiency syndrome, like rickets and the clarification of bones (Alli-Smith, 2009). The high calcium of *B.vulgaris* will ensure the 20 to 25 % of the daily requirement for calcium that aid strong bones and health teeth (Raghuvanshi & Singh, 2001). They are also required for growth and maintenance of bones, teeth and muscles (Dosunmu, 1997; Turan *et al*., 2003). Calcium and phosphorous are minerals present in largest quantity in the structure of the body and in the bone (Chionyedua *et al.*, 2009). Calcium in conjunction with phosphorus, magnesium, manganese, vitamins A, C and D, chlorine and proteins are all involved in bone formation.

Table 3. Result of the mineral analysis

Mineral content	Value (mg/100g)
Iron (Fe)	0.76 ±1.40
Magnesium (Mg)	18.60 ± 2.40
Copper (Cu)	0.08 ± 0.56
Sodium (Na)	73.60 ± 1.12
Potassium (K)	31.20 ± 0.46
Manganese (Mn)	0.86 ± 2.04
Calcium (Ca)	13.82 ± 0.98
Zinc (Zn)	0.29 ± 1.25

Valves are mean ± deviation of three replicate analyses.

Calcium is important in blood clotting, muscles contraction and in certain enzymes metabolic processes. Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting, and the regulation of cell permeability. It also plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system. It is very important that the normal calcium level in the diet should be balanced throughout life. Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves. In humans, Mg is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium. It is required in many enzyme-catalysed reactions, especially those in which nucleotides participate where the reactive species is the magnesium salt, e.g., MgATP2–. Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system. Sodium and potassium are required to maintain osmotic balance of the body fluid, the pH of the body, regulate muscle and nerve irritability and control of glucose absorption. Na and K take part in ionic balance of the human body and maintain tissue excitability.

Because of the solubility of salts, Na plays an important role in the transport of metabolites. K is of importance as a diuretic. Despite the fact that there is no recommended dietary allowance for potassium and sodium, it is recommended that the intake should be the same to counteract the effect of sodium in raising blood pressure. Beetroot may serve as good supplements in the body supply of magnesium, potassium, sodium and especially calcium - the daily requirement of calcium which have been put at 260 mg/day by (FAO/WHO, 2001). Copper is also a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron-oxidizing enzyme in blood (Mills, 1981). The observation of anemia in Cu deficiency may probably be related to its role in facilitating iron absorption and in the incorporation of iron into haemoglobin (FAO/WHO, 1974). Zinc is a component of many metalloenzymes, including some enzymes which play a central role in nucleic acid metabolism (Atukorala *et al*., 1987). In addition, Zn is a membrane

stabilizer and a stimulator of the immune response (Hambidge, 1978). Its deficiency leads to impaired growth and malnutrition (Prasad, 1981). Beetroot contains significant amounts of vitamins (Table 4) especially vitamin C (4.36 mg/100 g) one of the powerful natural anti-oxidants which helps body scavenge deleterious free radicals (one of the reasons for cancers development). The variability of the content observed for each nutrient and non-nutrient component analyzed, in comparison with earlier reports (Shyamala & Jamuna, 2010; McCance & Widdowson's, 1995; Bende & Bende, 1995), could be attributed to the variability in geographical location of the plants, the availability of soil nutrients, as well as species differences (Oko & Onyekwere, 2010). Vegetables are very important part of a diet. The data obtained from the analysis showed that *B. vulgaris* contain appreciable amount of proteins, fibre, carbohydrate, calorific value, and sufficient amount of mineral elements needed for normal body functioning, maintenance of the body, and reproduction.

Table 4. Result of the vitamin content

Vitamin content	Value
Vitamin A (µg/100g)	2.6 ± 0.50
Vitamin C (mg/100g)	4.36 ± 0.22
Vitamin E (mg/100g)	0.18± 1.34
Vitamin K (µg/100g)	3.20 ± 0.24
Vitamin B_1 (mg/100g)	0.034 ± 0.62
Vitamin B_2 (mg/100g)	90.053 ± 0.28
Vitamin B_3 (mg/100g)	0.352 ± 0.16
Vitamin B_6 (mg/100g)	0.03 ± 0.11
Panthotenic (mg/100g)	0.151± 1.60
Cholesterol (mg/100g)	0.04 ± 2.05

Valves are mean ± deviation of three replicate analyses.

Conclusion

It can therefore, be concluded that *B. vulgaris* can contribute significantly to the nutrients of man and animals and should be used as source of nutrients to supplement other major sources of nutrients. Vegetables have now become major components of human that should be eaten all year round. Since the fat content of *B. vulgaris* is low, it is recommended to those who are obese and those who are diabetic, since they do not contain high carbohydrate. It contain appreciable amount of minerals and is therefore, recommended to be consumed to supplement the daily requirement of Ca, K and Na recommended by FAO and WHO, respectively.

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