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Determination of nutritional contents and potential antioxidant activities of *Beta Vulgaris* L.

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Abstract

Beetroot (Beta vulgaris) is one of the vegetables that are commonly produced in the world.. Literature reveals that Beetroot extract has cardiovascular effect, antioxidant, anti-inflammation, and anti-tumor properties. The *antioxidant property of beetroot is thought to be a potent cancer-fighting agent because it also aids in the prevention of the development of malignant tumors and consider as a powerful agent to fight cancer. The aimed of this research is to assess the Phytochemical, proximate, minerals and antioxidant activities of Beetroot. The methanolic extract was subjected to phytochemical, proximate and minerals using standard methods. While the antioxidant (free radical scavenging activities) was cried out using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Phosphomolybdenum assay and Ferric reducing antioxidant power (FRAP).The proximate analyses results revealed a high moisture content of (72.7%), ash (14.7%), crude fibre (1.03%), fat (1.3%), protein (1.9%) and carbohydrate (8.3%). The phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, tannins and saponins. By using Atomic Absorption Spectroscopy (AAS), the following elements can be found: sodium (Na), potassium (K), iron (Fe), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), and calcium (Ca), in that order: 1.77, 8.50, 0.007, 0.003, 0.003, 0.13, 0.005, and 0.003) respectively. The antioxidant properties of Beta vulgaris L. demonstrated high radical activity at 20µg/ml in the following decreasing orders DPPH > Phosphomolybdenum > FRAP assays. The study has shown that Beta vulgaris L. contain appreciable amount of nutrients and potent antioxidant properties.*

Keywords: Antioxidant, AAS, Beta Vulgaris, Phytochemical, Proximate.

Introduction

According to Yen et al.[1], there are several health benefits associated with certain plants mainly due to the presence of bioactive substances found in them. These substances have targeted health importance ranging from angiogenesis inhibitory activities, antioxidative properties, antimutagenic, anticarcinogenic and atherosclerotic activities. Fortunately, the herbs family has been known to be a reservoir of important vitamins and other active compounds ranging from vitamin C, vitamin E as well as carotenoids. Herbs have also been reported to be reach in very important phenolic antioxidants compounds in the categories of phenolic acids[2], flavonoids and catechine[3].

Beetroot (Beta vulgaris L.) is a domesticated vegetable indigenously referred to as Shamandar and belongs to the family Amaranthaceae. Beetroot has been diversity in its usage for the treatment and management of various diseases and health challenges. Several researches have explored sundry properties including its carminative, homeostatic and especially protective functions of body organs[4,5]. According to Ninfali, P. and D. Angelino, D.[6] beetroot exhibit disease specific activities targeting specific ailments. The range of activities of beetroot extract cuts across antihypertensive, hepatoprotective, anti-inflammatory and important in regulating the level of glycemia in the body[7,8]. Extensive researches and studies have also elucidated on the on the ability of crude beetroot extract to shrink and suppress multi-organs tumors as well having a very potent anti-microbial activities against a wide range of microorganisms. The aphrodisiac properties of beetroot extract have also been highlighted in different literatures, beetroot was practically administered to stimulate sexual hormones and drive, it was extensively used to correct problems of sexual non-performance[9]. In the sphere of sports, beetroot has found a very important application. As against artificial stimulant for physical performance which are strongly prohibited in the sport administration, beetroot is proving a veritable alternative to chemical drugs and stimulant, simultaneously providing comparable outcomes[10].

Researchers over time has established the quantitative presence of phenolic compounds in the extract of beetroot, however, new frontiers are being open to find novel active substances they could have potential application in the food industry. The high level of interest in the extract of beetroot was primarily the exposition on the high level of anti-oxidant properties compared to other plants[11]. The strong anti-oxidant properties of beetroot have its origin in the presence of phenolic based compounds including flavonoids and catechins[12].

The need for food colourant with properties of water solubility and nontoxicity as well as bio-conformity is an ever need in the food industry. This problem is being addressed with a novel colourant called betalain isolated from the extract of beetroot. Betalain is finding wide range as a natural colourant because it is highly water soluble and non-toxic in biological systems[13].

Beetroot has also been discovered as a reservoir of extremely important compounds hitherto believed to be inherently present in vegetables. The presence of these active compounds in the juice of beetroot makes it a better alternative to large scale vegetable production and consumption[14]. Some of these compounds isolated thus far include basic mineral elements like potassium, sodium zinc, calcium, niacin, biotin, vitamins, soluble fibre etc.

With the drive to make consumables whole organic for health and safety reasons, beetroot is proving a formidable alternative to commonly used antioxidants to preserve the quality, taste, texture, colour and nutritional integrity of consumables[15]. This is more so, with studies having shown that traditional chemical-based antioxidant like butylated hydroxytolu (BHT) and butyrate hydroxyanisole (BHA) are in fact showing levels of toxicity at certain concentrations. This has opened a new vista of opportunity for the large-scale application of beetroot in the food industry and opening new business frontiers for the beetroot farmers.

The aim of this research work is to determine the phytochemical, minerals, nutritional contents and potential antioxidant activity of *Beta vulgaris* L.

Materials and Methods

Fresh beetroot was purchased from Sabon Gari market in Kano and washed thoroughly under tap water. The fruit was sliced into tiny pieces and allowed to air dry in the shade for six days. Using an electric blender, the chopped shade-dried fruit was ground into a powder and kept in airtight receptacles for later use. Using the maceration method, 50 grams of crushed powder were steeped in 250milliliters of methanol for 72 hours. Whatmann filter paper was used to filter the extract, a rotary evaporator was used to concentrate it and remove the extraction solvent (methanol), and the dry residue was stored in airtight bottles at 5°C until needed again.

Qualitative Screening of Phytochemicals: Alkaloids were detected (Wagner's Test) by dissolving the extract in diluted hydrochloric acid and filtering the mixture. Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) was used to treat the filtrate. Alkaloids are present when a brown or reddish precipitate is seen[16].

Detection of phenols by Ferric Chloride Test: Extract was treated with 3-4 drops of 5 % ferric chloride solution. The bluish black color formation indicates the presence of phenols[17].

Detection of flavonoids by lead acetate test: Few drops of lead acetate solution were added to the extract. The yellow color precipitate Formation indicates that flavonoids is presence[18].

Detection of Terpenoids by Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of green emerald color show the presence of diterpenes[19].

Detection of tannins (Gelatin Test): 1% gelatin solution containing sodium chloride was added to the extract. Formation of white precipitate indicates the presence of tannins[20].

Detection of saponins by Froth Test: Extracts was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins[21].

Proximate Analysis: AOAC[22] methods were used to determine the proximate analysis (moisture, ash, carbohydrate, lipids, protein, and fiber) of Beta vulgaris. The weight difference method was used to calculate the moisture and ash content. The difference method was used to calculate the amount of carbohydrates in the samples. The total amount of moisture, ash, protein, and fat content was deducted from 100 as follows:

100 – [Protein (%) + fat (%) + moisture (%) + ash (%)].

The Micro Kjeldahl method was used to determine a substance's nitrogen value, which is the precursor for protein. Protein was obtained by increasing the nitrogen value by a factor of 6.25.Using oven-dried samples from the moisture content measurement, crude fat was calculated.

Elemental Mineral Analysis of Beetroot: Using an atomic absorption spectrophotometer (AAS), Bulk Scientific model AVG 210, the sample was examined for mineral composition (sodium, calcium, potassium, magnesium, iron, copper, zinc, and manganese), as described by Shah[23], for plant samples. A weighted 2g processed sample was dry-asseshed at 550˚C in a Gallenkhamp muffle furnace using a cleaned porcelain crucible. After dissolving the resulting ash in 5 milliliters of HNO_3/HCl/ H_2O (1:2:3), the mixture was gradually heated on a hot plate until brown odors vanished. Five milliliters of de-ionized water were added to the remaining material in the crucible, and the mixture was heated until it became colorless solution. Each crucible's mineral solution was filtered using Whatman Grade No. 2 filter paper and then put into a 100 ml volumetric flask. The volume was adjusted with de-ionized water. Using an atomic absorption spectrophotometer (AAS), the solution was used for elemental analysis. Each element's concentration in the sample was determined using a 10cm long cell, and the results were expressed as mg/100g of dry matter, or as a percentage of the sample. A suitable standard solution was made for every mineral. It was possible to obtain the concentration vs. absorbance calibration curves.

Scavenging Activity of Beetroot Using DPPH Assay Methods: The DPPH test was used to measure the free radical scavenging activity[24]. Various concentrations of the extracts (20-100µg/mL) were taken and combined with 1mL of a 0.1mM DPPH solution in methanol. After half an hour, the setup was checked for absorption while kept at room temperature and in the dark. Absorbance was read at 517nm in spectrophotometer. The ability of the extract to scavenge DPPH radical was determined by the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

Scavenging activity of beetroot using FRAP assay methods: The extracts were diluted with 1mL of phosphate buffer (0.2 M, pH 6.6) and 1mL of 1% potassium ferricyanide at varying concentrations (20-100µg/mL). For twenty minutes, the mixture was incubated at 50ºC. 10% trichloroacetic acid, 1 milliliter, was added to the mixture. After adding 1mL of newly made ferric chloride (0.1%), the solution's absorbance was measured at 700 nm[25].

Scavenging Activity of Beetroot Using Phosphomolybdenum Assay: The reduction assay method, which is predicated on the creation of green phosphomolybdenum complex, was used to assess the antioxidant activity. One milliliter (mL) of reagent solution (consisting of 0.6 M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) was mixed with extracts at different concentrations. After being sealed, the tubes were incubated for ninety minutes at 95°C in a water bath. After bringing the samples down to room temperature, the mixture's absorbance at 695 nm was calculated in comparison to blank[26].

Results and Discussion

The results of phytochemical analyses were presented in Table-1. The results clearly show that beetroot methanolic extract contained secondary plant metabolites, however terpenoids were not detected. These metabolites included saponins, phenols, flavonoids, tannins, and alkaloids. These secondary metabolites have added to the plant's physiological activity and therapeutic usefulness[27]. In plants, phytochemical constituents are in charge of both pharmaceutical and poisonous properties[28]. They are applied therapeutically to treat injuries and a variety of illnesses[29]. Numerous purported consequences have been connected to their established roles as potent antioxidants, scavengers of free radicals, and metal chelators[30]. Alkaloids support the survival and fitness of plant species. They are employed as recreational and medicinal medications, and they frequently have pharmacological effects[31]. By producing a bitter taste, they deter insects from feasting on the leaves of plants. A variety of processing methods can be used to lessen the level of certain of these antinutrients[32]. On test animals, saponins have been shown to exhibit tumor-inhibiting activity[33]. Saponins have the ability to lower blood cholesterol and regulate cardiovascular disease in humans. Tannins have the potential to shield food proteins from microbial deterioration in the rumen[34].

Table-1: Result for Phytochemical Screening.

Compounds	Results
Alkaloids	Present
Phenols	Present
Flavonoids	Present
Tannins	Present
Terpenoids	Not detected
Saponins	Present

The results of mineral analyses were presented in ***T***able-2. Beetroot is abundant in potassium (K), sodium (Na), iron (Fe), and magnesium (Mg), with sodium having the largest content (Table-2). In general, the macronutrients are ranked in ascending order as follows: K > Na > Fe > Zn > Cu, > Ca, Mg, and Mn. It is well recognized that minerals are essential to living systems' physiology and metabolism[35]. To prevent metal deficiency condition, which includes rickets and bone discoloration, minerals must be included in supplemental meals[36]. B. vulgaris's high calcium content will guarantee that 20–25% of the daily need for calcium, which supports healthy teeth and strong bones, is satisfied[37]. The development and upkeep of bones, teeth, and muscles also depend on them[38,39]. The minerals calcium and phosphorus are found in greater concentrations in the body's structure, particularly in bone[40]. Calcium is involved in the development of bones in conjunction with phosphorus, magnesium, manganese, vitamins A, C, and D, chlorine, and proteins. Na is essential for the movement of metabolites because of the solubility of salts. K is significant since it is a diuretic. *Although there* is no recommended dietary allowance for potassium or sodium, it is advised that the consumption be equal in order to offset the effect of salt on blood pressure elevation. Beetroot can be a beneficial addition to the body's supply of magnesium, potassium, salt, and especially calcium, as the recommended daily intake of this mineral is 260 mg by *FAO/ WHO*[41]. Additionally, copper is a component of numerous enzyme systems, including lysyl oxidase, cytochrome oxidase and ceruloplasmin, bloods iron-oxidizing enzyme[42]. Cu's function in aiding iron absorption and incorporating iron into hemoglobin, maybe connected to the finding of anemia in cases of Cu deficiency[43]. Numerous metalloenzymes, including several that are essential to the processing of nucleic acids, include zinc[44].

Table-2: Results for mineral analysis.

Minerals	Value (ppm)
Sodium (Na)	1.77
Potassium (K)	8.50
Iron (Fe)	0.007
Magnesium (Mg)	0.003
Manganese (Mn)	0.003
Copper (Cu)	0.13
Zinc (Zn)	0.005
Calcium (Ca)	0.003

The results from the proximate analysis showed that, there is increase in the values of proteins, fats and oil, carbohydrates, dietary fibre and ash except for moisture content, compared to the results of the proximate analysis carried out by Vinson[45]. The difference in values might be due to different location of cultivation and other factors.

Table-3: Proximate analysis of *Beta vulgaris*.

Parameters	Values
Protein	1.88± 0.073
Fat	1.23± 0.122
Carbohydrate	8.46± 0.123
Crude fibre	1.0 ± 0.027
Ash	14.70 ± 0.096
Moisture	72.73± 0.125

Values are mean ± standard deviation of three determinations.

The Antioxidant Analysis's Results; Free radicals can be scavenged by antioxidant molecules before they cause damage to cells[46]. Human cells include extremely sophisticated enzymatic and nonenzymatic antioxidant mechanisms that cooperate to shield the body from free radicals. Antioxidants can be obtained exogenously (via diet) or endogenously (through dietary supplements)[46].

According to scientific research, food's antioxidant components have a significant protective effect on health. Regarding its antioxidant capacity, beetroot is ranked among the top ten vegetables, with a total phenol content of 50–60µmol/g dry weight[47]. The antioxidant obtained from this experiment showed that there is increase in the antioxidant activities compared to compare to the results of antioxidant activities of beetroot juice in the experiment carried out by Sheila[46].

Figure-1 reveals the antioxidant result of FRAP which shown that as the concentration increases, the % inhibition of free radicals also increases. The scavenging activity of the extract using phosphomolybdenum assay shows that, as concentration increases, the % inhibition of free radicals decreases seen Figure-2. The DPPH assay in Figure-3 followed the same pattern of increasing order, which is as concentration increases; the % inhibition of free radicals also increases.

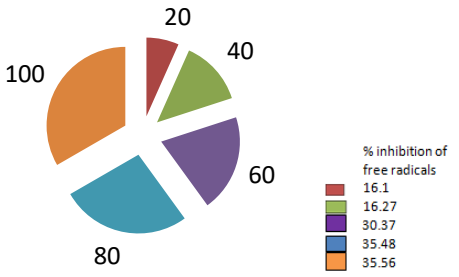

Figure-1: Scavenge activity of Beetroot using FRAP assay (µg/ml).

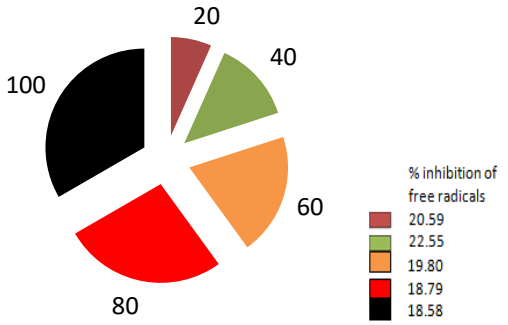

Figure-2: Scavenge activity of Beetroot using phosphor molybdenum assay (µg/ml).

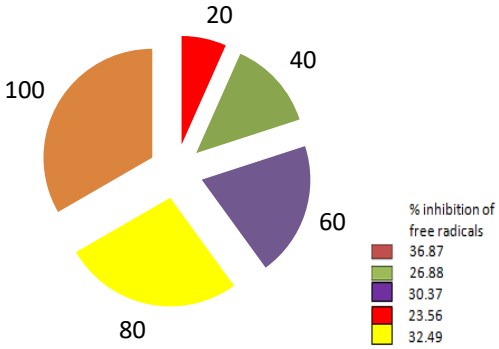

Figure-3: Scavenge activity of Beetroot using DPPH assay (µg/ml).

Conclusion

The natural foods we consumed contain phytochemical components that may help reduce the risk of developing significant health disorders. Beta vulgar is L. is a plant that is high phytochemicals, minerals and nutrient and can also contribute significantly to the nutritional requirement of human. It can also be use pharmacologically due to the presence of active compounds.

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