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Chemical Analysis and Biological Activity of Natural Preservative from Beet root (*Beta vulgaris*) Against Foodborne Pathogens and Spoilage Organisms.

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ABSTRACT: Proximate and elemental analysis of *Beta vulgaris* was carried out using standard procedures. Ethanolic and aqueous extract of the root were analysed qualitatively and quantitatively for phytochemicals. The agar well diffusion assay was employed to determine the antibacterial activity of both ethanolic and water extracts of *Beta vulgaris*. Carbohydrate, protein, fat/oils, ash and moisture content were 9.50±0.01, 1.58±0.00, 0.23±0.00, 1.43±0.01 and 88.69±1.20 % respectively. The mineral content ranged from 1.90±0.01 to 15.98±0.01 % with calcium being the lowest and manganese the highest. Phytochemicals analysis showed the presence of tannins (6.00mg/100 g), saponins (3.60 mg/100g), alkaloids (127.60 mg/100g), flavonoids (6.40 mg/100g), glycosides (0.651 mg/100g), steriods (16.30 mg/100g) and terpenoids (116.30 mg/100g). Antimicrobial activity test showed that the ethanol extract was more effective than the aqueous extract. The most susceptible organisms were *Bacillus subtilis* (MIC, 19.5 µg/ml) among the Gram-positive bacteria and *Escherichia coli* (MIC, 39.1 µg/ml) among the Gram-negative food-borne pathogens. Beet root extract with its broad spectrum biologic acttivity can be harnessed as a food preservative.

Keywords: Proximate, elemental, composition, antibacterial, preservative

Introduction

Plants are primary source of medicines, fiber, food, shelters and other items in everyday use by humans with roots, stems, leaves, flowers, fruits and seeds providing food for humans. 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 (Okwu and Josiah, 2006). *Beta vulgaris* known as garden beet or red beet in the US scientific literature and beetroot in Europe and in many other countries is a herbaceous biennial or rarely perennial plant. It is best known in its numerous cultivated varieties, the best of which is the purple root vegetable known as the beetroot or garden beet (Chionyedua *et al*., 2009).

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 (Chionyedua *et al*., 2009).

Beetroot is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which are composed of two main groups, the red betacyanins and the yellow betaxanthins. In ancient times, beetroot was believed to help enhance human sex hormones and as an aphrodisiac. The juice of beetroot is also consumed as a natural remedy for sexual weakness and to expel kidney and bladder stones (Hunell, 2003). Beetroot (*Beta vulgaris*) is the taproot portion of the beet plant, also known as the table beet, garden beet, red or golden beet, or informally simply as the beet. Beetroot is native of Southern Europe, it has a vibrant crimson colour which comes from pigments no other vegetable has. Reports revealed that it ranks among the ten most powerful vegetables with respect to its antioxidant and antibacterial capacity (Winkler *et al*., 2005).

It is a member of the Chenopodiaceae plant family, which encompasses a diverse range of both economically important species as well as numerous agricultural weeds. *Beta vulgaris* is the species of greatest economic importance within the genus *Beta*. This species is further divided into four important cultural types: sugar beet, fodder beet, Swiss chard and beetroot, each of which has been developed for a particular use. These plants are indigenous to the Mediterranean region and western and eastern Europe. *Beta vulgaris* is either annual or biennial in its reproductive habit. Seed may be produced in the first year after planting or an extended period (50-120 days) of cool weather may be required to stimulate flowering. Seed is produced in regions with relatively low temperatures (0^0-10^0C). It is several of the cultivated varieties of beet grown for their edible taproots and their greens. These varieties have been classified as *B. vulgaris* subsp. *vulgaris* (Singh and Hathan, 2014).

In street markets, indoor markets, and fruit and vegetable distribution centers, the leaves of beet root are cut off from the bulb to be used as organic fertilizer and animal feed or are discarded into the environment as waste. Beetroots have long been used for medicinal purposes, primarily for disorders of the liver as they help to stimulate the liver's detoxification processes (Rauha *et al*., 2000). The plant pigment that gives beetroot its rich, purple-crimson colour is betacyanin. Betacyanin is a powerful agent, thought to suppress the development of some types of cancer. Beetroot is rich in fibre, exerting favourable effects on bowel function, which may assist in preventing constipation and help to lower cholesterol levels too (Reddy *et al*., 2005). Beetroot fibre has been shown to increase the level of antioxidant enzymes in the body, specifically glutathione peroxidase, as well as increase the number of white blood cells, which are responsible for detecting and eliminating abnormal cells. Beets are also one of the richest sources of glutamine, an amino acid, essential to the health and maintenance of the intestinal tract. Other studies have looked at the effect of beetroot juice on blood pressure. A reduction in blood pressure is beneficial for the avoidance of heart disease and stroke. Studies state that nitrate rich foods like beetroot may help in heart attack survival (Rauha *et al.,* 2000).

Beetroot contains some fiber, potassium and B-group vitamins particularly folate. It also contributes a small amount of iron and Vitamin C. Beetroot contains significant amounts of vitamins, 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). Beet root is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which comprise two main groups, the red betacyanins and the yellow betaxanthins. Betalains have been extensively used in the modern food industry (Stintzing and Carle, 2004). Betalains, because of their relative scarceness in nature, have not been much explored as bioactive compounds. In some countries all parts of the beetroot are regularly eaten. It is one of the few vegetables that are consumed more frequently pickled than in other ways. Baby beetroot leaves may be found in salad mixes. Several varieties are commonly available with roots varying in shape from round to spherical. Other than as a food, beets have use as a food coloring and as a medicinal plant.

Colours are important quality indicators that determine the consumer acceptability of foods. *Beta vulgaris* has been reported to be composed of different pigments all belonging to the class betalains. Betalains are water-soluble nitrogen-containing pigments, found in high concentrations in *Beta vulgaris*. The use of betalains as food colorant is approved by European Union and betalains are labeled as E-162. Betalains are more stable to pH and temperature and exhibit broad pH stability which are suited for low-acid foods where coloring with anthocyanins is usually not possible (Stintzing and Carle, 2004). Betalain pigment mixtures can be used as a natural additive for food, drugs and cosmetic products in the form of beet juice concentrate or beet powder (Dörnenburg and Knorr, 1996).

Plant metabolites represent an important source of sugars, minerals, organic acids, dietary fibre, as well as bioactive compounds like phenols, alkaloids and flavanoids which are synthesized as secondary metabolites and are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Turan *et al.,* 2003). These secondary metabolites produced by plants have been reported to possess a wide range of chemical, physical and biological activities.

Betalains is a component of beet root which consist of two sub- classes: betacyanins (red-violet pigments) and betaxanthins (yellow orange pigments) (Stintzing and Carle, 2004). They have been reported to have antimicrobial and antiviral effects and also can inhibit the cell proliferation of human tumor cells (Stintzing and Carle, 2004). This

study seeks to determine the proximate, elemental composition and characterize the Phytochemical profile and determine the antibacterial activity of *Beta vulgaris* extract for possible usage as a food preservative.

Materials and Methods

Sample Collection and Preparation

Beetroot *(Beta vulgaris)* were purchased at Airport road vegetable market, Benin City, Nigeria and authenticated at the Department of Botany, University of Benin, Benin City. Dirt and sand were removed from the roots by rinsing in clean water. They were sun-dried for six days and pulverized using an electric blender into a fine powder of 60 mesh sieve size. The powdered material was stored in air tight jars in a refrigerator at 4 °C until used for analysis.

Extract Preparation

The method of Omogbai and Eze (2011) was used with some modifications. Two extractants; water and ethanol (95%) were employed for the phytochemical extraction.

(a) For extraction with water, 90 g of powdered beetroot was dissolved in 900 ml sterile distilled water in a 1L Erlenmeyer flask. The mixture was kept undisturbed at room temperature for 72 h in a sterile flask covered with aluminium foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. The filtrate was evaporated with a water bath leaving a solid mass of crude extract.

(b) For extraction with ethanol, the procedure was the same as used for aqueous extract. The filtrate was evaporated at a low pressure with a Buchi Rotavapor R-200 at 45 ^{0}C leaving a solid mass of crude extract.

Qualitative Phytochemical Analyses

Qualitative phytochemical analyses were carried out according to standard methods. To 1 ml of the extract was added 2 ml of acetic acid and then cooled in an ice bath at 4^{o}C. To the mixture 1 ml of concentrated tetraoxosulphate acid (H_2SO_4) was added drop wise. The formation of an oily layer on top of solution indicated the presence of glycosides (Odebiyi and Sofowora 1978). To 3 ml of the extract was added 1ml of 1 % HCl. This resulting mixture was then treated with few drops of Meyer's reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids (Ogukwe *et al*., 2004). Five drops of olive oil was added to 2 ml of the beet extract and the mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins (Trease and Evans 1996). Two drops of 5% $FeCl_3$ was added to 1 ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins (Trease and Evans 1996). To 1 ml of the extract was added 3 drops of ammonical solution (NH_3^+) followed by 0.5 ml of concentrated HCl. The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids (Odebiyi and Sofowora 1978). To 1 ml of the plant extract was added 1ml of concentrated tetraoxosulphate acid (H_2SO_4). A red colouration confirmed the presence of steroids (Trease and Evans 1996).

Quantitative Phytochemical Analyses

Alkaloids Determination

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

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 to obtain a constant weight.

Cyanogenic Glycoside Determination

A portion (5 g) of the sample was made into a paste and the paste was dissolved in 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 the absorbance was recorded at 500 nm. The cyanide content was extrapolated from a cyanide standard curve.

Saponin 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 °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 °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 was added. The combined n-butanol extracts was washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponins content was calculated as a percentage.

Tannins Determination

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 pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Proximate and Mineral Analysis

The protein content of samples was determined using Kjeldahl method with Nessler's reagent. The ash content of each sample was determined by dry-ashing in a muffle furnace at 600 ^{0}C (AOAC, 2000). Moisture content of each sample was determined by the gravimetric method (AOAC, 2000) by drying the sample to constant weight at 105 ^{0}C. The sample was weighed before and after drying. An Atomic Absorption Spectrophometer (Thermo Electron S-series U.S.A.) was used in quantitative trace mineral analysis of samples. The elements were determined by flame atomic absorption with detection limit of 1ppm (AOAC, 2000).

Test Microorganisms

Gram negative bacteria such as *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa, Salmonella typhimurium* and *Proteus mirabilis* used for the determination of antibacterial activity were obtained from the Department of Medical Microbiology Laboratory of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Gram-positive organisms used include *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus, Listeria monocytogenes and Enterococcus faecalis.* They were laboratory stock cultures from chicken and beef and obtained from Lahor Research and Diagnostic Laboratories, Benin City, Nigeria. Phenotypic profiling of both Gram-positive and Gram-negative bacteria was undertaken using API 50CHB and API 20E strips (BioMerieux, Marsielle, France) respectively. The microbial cultures were maintained on nutrient agar slants at 4 ºC.

Antibacterial Activity Testing of Beetroot

The bacteria were inoculated in 10 ml of sterile nutrient broth in conical flask, and incubated overnight at 37 ºC in a rotatory shaker. The culture was swabbed on the surface of sterile Mueller Hinton agar (Hi-media) plate using a sterile cotton swab. Agar wells were prepared with the aid of sterilized cork borer with radius 5 mm using a micropipette. Concentrations (9.77 µg/ml to 2500 µg/ml) of beetroot extract were prepared using the extraction solvents. The extracts were filter sterilized through 0.22 µm membrane. Aliquots of 100 µl of the extracts of beet root were added to each well of the plate. The plates were incubated in an upright position at 37 ºC for 24h. Distilled deionised water and ethanol were used as control. The diameter of inhibition zones measured in mm were recorded.

Minimum Inhibitory Concentration (MIC) Assay

The experiment was carried out using the two fold serial dilution method described by Omogbai and Ahonsi (2013) and Cheesbrough (2000). The stock test extract solution was prepared at concentration of 2500 µg/ml in nutrient broth and serially diluted to obtain 1250, 625, 312.5, 156.25, 78.13, 39.10, 19.5 and 9.77 µg/ml. Thus equal volume of the beetroot extract and nutrient broth (2 ml) was dispensed into sterile test-tubes. a 0.1 ml of standardized inoculum (1.5×10^6) cfu/ml was added to each tube. The control tube had only broth and inoculum

without the extract. The inoculated tubes were incubated at 37±1 °C for 24 h for bacterial assay. After the incubation period, tubes were removed and observed for any turbidity in the solution. The lowest concentration of the extracts which inhibited bacterial growth by showing no turbidity was recorded as the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration

This was carried out as an adjunct to the MIC test and employed to ascertain the minimal concentration of the extract that is lethal to the target microorganism *in-vitro*. In this procedure sterile Muller-Hinton agar plates were inoculated with samples from each of the test-tubes showing no visible growth from the MIC test. The plates were incubated at 37 °C for 24 h. The least concentration of extract that showed no growth was taken as the minimum bactericidal concentration (Cheesbrough, 2000).

Results

In Table 1 is shown the proximate and mineral composition of beetroot. The highest value for the proximate composition was 88.69±1.20 % for moisture content while the least of 0.23±0.00 % was for fat/oils. The mineral content was found to have the least value of 1.90±0.01 % calcium and 15.98±0.01 % for manganese as the highest. The results of phytochemical analysis of *Beta vulgaris* revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, cyanogenetic glycosides and steroids in the ethanol extract. The aqueous extract only contained alkaloids and flavonoids (Table 2). The quantitative phytochemical analysis of *Beta vulgaris* showed that constituents had different concentrations (Table 3). Glycosides had the least concentration of 0.65±0.01 mg/100g while alkaloids had the highest concentration of 127.60±1.50 mg/100g from the ethanol extract. The concentrations of the secondary metabolites were considerably low or even absent in the water extract. Alkaloids and flavonoids were 50.15±0.05 mg/100g and 1.60±0.01 mg/100g compared to 127.60±1.50 mg/100g and 6.40±0.01 mg/100g from the ethanol extract.

Table 1: Proximate and Mineral analysis of Beet root (*Beta vulgaris*)

Proximate/Mineral	Composition (%)
Carbohydrate	9.50±0.01
Protein	1.58±0.00
Fat/Oil	0.23±0.00
Ash	1.43±0.01
Moisture	88.69±1.20
Magnesium	5.81±0.18
Calcium	1.90±0.01
Phosphorus	5.97±0.03
Sodium	5.10±0.00
Potassium	7.03±0.01
Zinc	3.96±0.10
Manganese	15.98±0.01
Iron	5.94±0.06

Values are means of five (05) samples and standard deviation from the mean.

Table 2: Qualitative phytochemical analysis of *Beta vulgaris*

Constituents	Ethanol extract	Water extract
Tannins	+	-
Saponins	+	-
Flavonoids	+	+
Terpenoids	+	-
Glycosides	+	-
Steriods	+	-
Alkaloids	+	+

+ = PRESENT, – = ABSENT

Table 3: Quantitative Phytochemical Analysis of *Beta vulgaris*

Constituent	Ethanol extract (mg/100g)	Water extract (mg/100g)
Tannins	6.00 ± 0.10	0.00±0.00
Saponins	3.60 ± 0.50	0.00±0.00
Alkaloids	127.60 ± 1.50	50.15±0.05
Flavonoids	6.40 ± 0.01	1.60±0.01
Glycosides	0.651 ± 0.01	0.00±0.00
Steriods	16.30 ± 0.20	0.00±0.00
Terpenoids	116.30 ± 0.20	0.00±0.00

NB: Values are means ± standard deviation from three replicate analyses

The susceptibility profile of food-borne pathogens and spoilage organisms to ethanol and aqueous extracts of beetroot are shown in Tables 4 and 5. The zones of inhibition shown by the organisms were considerably larger for the ethanol extract compared to the water extract. At 1250µg/ml, the zones of inhibition produced by *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi* with ethanol extract were 28, 21, 21, 26, and 23 mm respectively compared to 15,11, 10, 9, and 14mm with the water extract. Of all the microbial pathogens tested, *Listeria monocytogenes* was not susceptible at all concentrations used. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the extracts is presented in Table 6. In general the Gram-positive organisms with the exception of *Listeria monocytogenes* showed lower MICs and MBCs. With ethanol extract, *Bacillus subtilis* showed the least MIC and MBC of 19.5 µg/ml and 39.1 µg/ml respectively. The bacteria *Enterococcus faecalis* and *Pseudomonas aeruginosa* had the highest MBC of 1250 µg/ml. While the ethanol extract showed lower MICs and MBCs, the aqueous extract depicted higher values in the order of 625 µg/ml to greater than 2500 µg/ml (Table 6).

Table 4: Susceptibility of Ethanolic Beetroot Extract against Foodborne Pathogens and Spoilage Organisms

Concentration (µg/ml)	Zone of Inhibition against test organisms (mm)									
	Staphylococcus aureus	***Bacillus cereus***	***Listeria monocytogenes***	***Bacillus subtilis***	***Enterococcus faecalis***	***Pseudomonas aeruginosa***	***Escherivhia coli***	***Salmonella typhimurium***	***Klebsieella pneumoniae***	***Proteus mirabilis***
2500	30	21	13	23	25	21	28	25	28	27
1250	28	21	0	21	10	18	26	23	24	25
625	24	18	0	20	7	17	25	20	22	24
312.50	20	17	0	20	0	14	23	16	18	21
156.25	18	15	0	18	0	13	22	15	15	18
78.13	17	0	0	15	0	0	20	13	12	15
39.10	16	0	0	13	0	0	14	0	0	0
19.50	0	0	0	10	0	0	0	0	0	0
9.77	0	0	0	0	0	0	0	0	0	0

Table 5: Susceptibility of Aqueous Beetroot Extract against Foodborne Pathogens and Spoilage Organisms

Concentration (µg/ml)	Zone of Inhibition against test organisms (mm)									
	Staphylococcus aureus	***Bacillus cereus***	***Listeria monocytogenes***	***Bacillus subtilis***	***Enterococcus faecalis***	***Pseudomonas aeruginosa***	***Escherivhia coli***	***Salmonella typhimurium***	***Klebsieella pneumoniae***	***Proteus mirabilis***
2500	20	13	0	15	10	9	13	17	16	20
1250	15	11	0	10	0	0	9	14	8	15
625	10	0	0	0	0	0	0	12	0	11
312.50	0	9	0	0	0	0	0	8	0	9
156.25	0	0	0	0	0	0	0	0	0	0
78.13	0	0	0	0	0	0	0	0	0	0
39.10	0	0	0	0	0	0	0	0	0	0
19.50	0	0	0	0	0	0	0	0	0	0
9.77	0	0	0	0	0	0	0	0	0	0

Table 6 : Minimum Inhibitory and Bactericidal Concentration of *Beta vulgaris* Extract on Food borne pathogens and Food Spoilage organisms

Microbial pathogens	Ethanol Extract (µg/ml)		Aqueous Extract (µg/ml)	
	MIC	MBC	MIC	MBC
Staphylococcus aureus	39.10	78.13	625	625
Bacillus cereus	156.25	156.25	1250	2500
Listeria monocytogenes	2500	>2500	>2500	>2500
Bacillus subtilis	19.50	39.10	1250	2500
Enterococcus faecalis	625	1250	2500	>2500
Pseudomonas aeruginosa	156.25	1250	2500	>2500
Escherichia coli	39.10	156.25	1250	>2500
Salmonella typhimurium	78.13	312.50	625	1250
Klebsiella pneumoniae	78.13	156.25	1250	>2500
Proteus mirabilis	78.13	312.50	312.50	1250

Discussion

The result of the nutritional composition of *Beta vulgaris* showed it contains a lot of water and minute amount of oil. The protein and carbohydrate content shows it to be low in calories which make beetroot a good fasting food. However beetroot is rich in minerals which enhance its nutrition. Minerals play a vital role in the maintenance of human health. Sufficient evidence is now available to confirm that certain minimum amount of minerals is desirable since their deficiency have many negative health effects: diseases and possible aggression from toxic elements and bacteria (Amato, 1999). The present study reveals an array of minerals in *Beta vulgaris* with manganese being the most abundant followed by potassium. Magnesium, phosphorus, iron, sodium and zinc were present in modest amounts while calcium was in minute quantity. Manganese is essential in bone formation, act as a co-factor for the enzyme prolidase necessary for collagen- a structural skin component. Besides it is required by multiple enzymes in gluconeogenesis and as a co-factor in manganese superoxide dismutase (MnSOD), functions as an antioxidant against oxygen-related and UV light damage of skin cells and other cell types (Abdalian *et al*., 2013).

Health-wise, potassium and sodium are macronutrients required for the maintenance of cellular water balance, acid-base balance and nerve transmission (Wardlaw, 1999). Deficiencies of these macronutrients can lead to muscle cramps mental confusion, loss of appetite and irregular cardiac rhythm. However, there is a direct relationship between sodium intake and hypertension in humans (Dahl, 1972) therefore it is desirable that the concentration of sodium in food is not high as that of potassium. The results therefore indicate a desirability of the consumption of beetroot since the concentration of potassium is higher compared to that of sodium. The mineral magnesium is effective for muscle function, calcium for healthy bones and teeth while phosphorus is a component of many enzymes and nucleic acids. Iron is important in blood formation while zinc enhances immunity and reproductive function (Hunt *et al*, 1980, Wardlaw, 2000). Thus owing to the nutritional and mineral composition, beetroot can make a major input to a diet.

The result of Phytochemical analysis indicated that the root of *B. vulgaris* is rich in phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, Steriods and Terpenoids. The presence of these secondary metabolites has been reported by Omogbai and Eze (2010) to contribute to the medicinal value as well as physiological activity in plants. Saponins have been reported to show tumor inhibiting activity on experimental animals (Akindahunsi and 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. Flavonoids and alkaloids have been shown to possess antibacterial, anti-inflammatory antiviral and anti-

neoplastic functions. This is due to their strong anti-oxidant, free radical scavenging action and as metal chelators (Mishra *et al*., 2008).

The ability to produce a safe food product with extended storage life which is acceptable to the consumer according to the relevant food standard guidelines is the objective of food preservation. This is achieved through designing processing steps specific to different products. The goal is to combine a range of processes, for example mild heat stress and a low concentration of antimicrobial preservatives to give a safe and quality food product (Tajkarimi *et al*., 2010). Plant antimicrobials which are phytochemicals fulfill the needs of today's consumer looking for wholesome food without chemical preservatives. These can be classified as novel compounds obtained from plants that delay microbial growth of pathogens and spoilage organisms in food (Roller, 2003).

Balasundram *et al*. (2006) reported that phenolic compounds are one of the most widely occurring phytochemicals in plants. They contribute to the sensory properties when added to food and have antioxidant and antimicrobial properties, characteristics that are useful in extending the shelf-life of food. Flavonoids are classified under phenolic groups in plants which have been known to possess antimicrobial activity. The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism. Related studies of antimicrobial activity indicate that crude extracts containing flavonoids, triterpenes and steroids have showed significant activity against various strains of *Staphylococcus aureus, Streptococcus faecalis* and *Escherichia coli* (Chattopadhay *et al*., 2001; Figueroa-Valvere *et al*., 2011).

The alkaloid rich fraction obtained from various parts of some plants such as *Prosopis juliflora* have been shown to exhibit antibacterial property using disc diffusion method on several Gram-negative and Gram positive bacterial strains like *E.coli, Staphylococcus aureus, Bacillus cereus, Psuedomonas putida, Klebsiella, Salmonella, Acinetobacter and Alcaligenes* (Ozcelik *et al*., 2011; Singh *et al*., 2011). This is in consonance with the present study. No doubt therefore the presence of phytochemicals in beetroot extract is important for both pharmacological and food preservative purposes as a natural antimicrobial.

The ethanolic extract of beet root showed more antibacterial activity compared to the aqueous extract. This activity may be due to the fact the ethanol extract contained more of the phytochemical constituent due to the inherent solubility properties of these phytochemicals in the organic solvent. Plant extracts from organic solvents have been found to give higher antimicrobial activity compared to water extract. Furthermore, water soluble flavonoids (mostly anthocyanins) have been reported to lack antimicrobial significance (Das *et al*., 2010).

The MIC recorded in this study varied proportionately to the susceptibility patterns of the bacteria species against the active principle in the plant extract. The most susceptible organisms to beetroot extract were *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli.* These organisms are common spoilage organisms of foods. *Staphylococcus aureus* in particular is known to cause food poisoning. *Escherichia coli* depending on the species can apart from spoilage also result in disease conditions in humans. Of interest in this study is the ability of beetroot extract to curtail their growth with low MICs of 19.5 µg/ml and 39.1 µg/ml. The low minimum bactericidal concentration (MBC) recorded in this analysis for Gram-positive bacteria invariably means they are more susceptible to the extract compared to the generally higher MBCs of the Gram-negative organisms. But the overall effect is that both kinds of bacteria were killed even at higher concentrations of the extract which will make it quite useful for food preservative purposes instead of synthetic chemicals.

Conclusion

This study shows that the ethanol extract of beetroot has good antimicrobial activity against gram-positive and gram-negative organisms. Importantly and in practical terms, beetroot extracts can be harnessed and used in the food industry as a natural antimicrobial food preservative owing to the bioactive phytochemical content. The proximate and mineral content justifies why beetroot is recommended as a vegetarian diet that could improve human health.

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