



Research Article

Phytochemical, Nutritional, Toxicity and Antihypertensive Properties of Methanol Root Extract of *Beta vulgaris* Lam

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ABSTRACT

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The present study was carried out to investigate the phytochemicals and also analyze the antihypertensive effects of *Beta vulgaris* with a view to determining the efficacy of the plant as an antihypertensive. Five kilograms (5 kg) of *Beta vulgaris* were exhaustively extracted using the cold infusion method (maceration) with 85% methanol. The specimen was analyzed for proximate and elemental contents. The extract of *Beta vulgaris* was phytochemically screened and evaluated for its toxicity and antihypertensive effect using Wistar strain rats. The results of proximate content revealed that the percentages of moisture, fibre, protein, ash, dry matter, and carbohydrates were 82.72, 5.83.00, 6.21, 2.00, 1.00, 17.28, and 2.25%, respectively. Micro and macro elements such as Fe (0.08), Cd (0.03), Mn (1.37), Zn (0.09), Pb (0.18), Na (2.35), and anions NO₃²⁻(4.90), SO₄²⁻(19.00), and PO₄²⁻(4.20) were determined using an atomic absorption spectrophotometer (AAS). The crude extract was screened for the presence of phytochemicals using standard methods and revealed the presence of flavonoids, terpenoids, steroids, tannins, cardiac glycosides, and carbohydrates. The acute toxicity level was determined by Lorke's method, and the crude extract was considered safe with an oral LD₅₀ of 3807.8 mg/kg bwt in rats. The crude extract of *Beta vulgaris* significantly reduced (p<0.05) blood pressure and heart rate in hypertensive rats in dose-dependent manner when compared with the standard antihypertensive drug. The highest reduction was observed on day seven with reductions of systolic and diastolic blood pressures, MAP and HTR, 132±2.44, (26.26), 85±1.6, 109.2±2.44, 70.20±1.67 at the dose of 200 mg/kg. Therefore, the folkloric claim for the use of this plant, *Beta vulgaris*, as a remedy to hypertension has scientific support, as examined.

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INTRODUCTION

The use of plants as medicine is an ancient practice practiced by all societies, particularly the indigenous peoples. This practice is still practiced in developing countries. It is on this basis that researchers keep on working on medicinal plants to produce and develop the best for physiological uses as medicines (Usman and Osuji, 2007). Man uses plants as food, clothing, fuel, shelter, and the most useful necessity of life, which is the maintenance and management of different diseases (Mustapha, 2013). The majority of the rural population in Africa still employs traditional methods. This usage is attributed to its ease of access and affordability to the people who live mostly in poor rural communities. Such traditional medicines often are herbs and other plant materials whose efficacy and safety has a very wide claim (Adzu *et al.*, 2004), and the state of medicinal plants research has been emphasized in many developing countries (Edeoga *et al.*, 2005).

The folklore system of medicine consists of enormous number of plants with a variety of medicinal, pharmacological and therapeutic importance and therefore represents a priceless reservoir of novel bioactive ingredients (Mustapha, 2013). Interest in the traditional systems of medicine and herbal medicine has increase substantially in both developed and underdeveloped countries over the past two decades. The medicinal value of these plants depends on some chemical compounds that produce a definite physiological action in the human body (Zaku *et al.*, 2009). These properties are often attributed to the important phytochemical constituents like triterpenes, anthocyanins, coumarins, flavonoids, alkaloids, etc. Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficiencies of many modern drugs used for the control of many infections as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drug, for the

maintenance of personal health (Levy, 1998; Van den Bogaard *et al.*, 2000; Smolinski *et al.*, 2003).

Hypertension is a growing public health concern in sub-Saharan countries where it was previously not reported. One in three adults worldwide has hypertension (HTN) and the prevalence has been shown to increase with age (Benzie and Wachtel, 2011). Hypertension (HTN) or high blood pressure (BP) is a chronic medical condition in which the blood pressure in the arteries is elevated. It is classified as either primary or secondary. About 90 to 95% of cases are termed primary HTN, which refers to high BP for which no medical cause can be found (Carretero and Oparil, 2000). The remaining 5 to 10% of cases, called secondary HTN, are caused by other conditions that affect the kidneys, arteries, heart, or endocrine system (Beevers, *et al.*, 2001). Persistent HTN is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure (Pierdomenico *et al.*, 2009) Moderate elevation of arterial BP leads to shortened life expectancy. Dietary and lifestyle changes, as well as medications, can all help to improve blood pressure control and reduce the risk of associated health complications. The systolic and diastolic blood pressures are typically used to classify HTN. Systolic BP is the BP in vessels during a heartbeat. Diastolic BP is the pressure between heartbeats. Pre-HTN or HTN is defined as a systolic or diastolic blood pressure measurement that is higher than the accepted normal values for the individual's age. Several classes of anti-hypertensive medications have been developed with the aim of reducing BP and consequently the associated risks (De Wet *et al.*, 2012).

There are several varieties in the Chenopodiaceae family with bulb colours ranging from yellow to red of which Beetroot (*Beta vulgaris*) [Figure 1] is a classified. Deep red beet roots are the most commonly consumed by humans, both cooked and raw as

salad or juice. Natural food colors are gaining popularity as synthetic colors become more prevalent as consumers are increasingly critical of dyes. However, betalains are less commonly used in food processing than anthocyanins and carotenoids, despite the fact that these water-soluble pigments are stable between pH 3 and 7. White beet juice has been used for cleansing digestive quality to "open obstructions of the liver and spleen—good for

headache and all affections of the brain. The juice is also good for "blisters and blains of the skin and as a decoction in water and vinegar cleanses the head of dandruff and relieves running sores and ulcers (Bourn and Prescott, 2002). White beet juice can also help prevent baldness and hair shedding. Red beet juice is known to relieve yellow jaundice, and when placed in the nostrils, it relieves ringing in the ears and toothaches.



Figure 1: Beetroot (*Beta vulgaris*) plant

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant sample was collected from vegetable farm (Kuru Karami) at Bukuru town in Jos South local Government Area, Plateau State, Nigeria. The sample was transported in a clean plastic bag, identified and authenticated by a plant taxonomist in the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. Sample voucher specimen (9900B) was deposited at the Postgraduate Research Laboratory of the Department of Pure and Applied Chemistry, University of Maiduguri, Borno State. The plant sample was cleansed, free from foreign material through manual hand picking, mash using a wooden mortar and pestle, and kept in the laboratory under shade at room temperature until required for analysis.

Proximate Content of *Beta vulgaris* (Beetroot)

The proximate analysis was carried out in line with the method described by the Association of Official Analytical Chemists (AOAC, 1990) for the estimation of crude fibre, crude carbohydrate, crude protein, carbohydrate content, crude fiber, dry matter, ash content, and moisture content etc.

Elemental Content Analysis

Sample Digestion and Preparation for Analysis were carried out in line with the standard procedure by Radojevic and Bashkin (1999).

Analysis of Micro and Macro Elements in *Beta vulgaris* Root

The macro and microelements were determined using a Perkin-Elmer Analyst 300 single-beam analyzer. The data from the Atomic Absorption Spectrophotometer (AAS) were obtained in parts per million (ppm)

values were converted to mg/g values. Working calibration curves were used to create calibration curves for each element. The laboratory procedures outlined by Radojevic and Bashkin (1999) for plant macro- and microelement preparation and determination were used.

Extraction of Plant Sample

A mass of 5 kg of pulverized *Beta vulgaris* root was extracted exhaustively by maceration method for seven days using 85% methanol as solvent. The extract was concentrated to dryness at reduced pressure at a temperature between 35 °C to 45 °C. The extract was weighed, labeled and kept for further analysis.

Phytochemical Screening of Extract

The crude methanol extract of *Beta vulgaris* extract was analysed for the presence of phytochemicals, which include flavonoids, terpenoids, tannins, cardiac glycosides, saponins, carbohydrates amongst other, using the already established procedures by Harborne (1973), Brain and Turner (1975), Markham, (1987) Vishnoi (1979), Silva *et al.* (1998) and Evans (2009).

Experimental Animals

All the experiments performed on laboratory animals in this study were carried out based on standard procedures. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals, CIOMS and ICLAS, (2012).

$$LD_{50} = \sqrt{a \times b}$$

Where a = least dose that killed a rat

b = highest doses that did not kill a rat

Antihypertensive Study

Sixty (60) albino rats of both sexes weighing between (180 - 250 g) were anaesthetised with pentobarbitone sodium (1 mg/kg) intraperitoneally. The jugular vein was cannulated for administration of drugs, while the carotid artery was cannulated and connected via CODA II, NIBP Recording system (Kent Scientific Co-operation) were used to measure blood pressure SBP, DBP, MAP and HR of the experimental animal (rats). The animal's body temperatures were

A total of eighty-four (84) Wistar stain rats (100-180 g) of both sexes were purchased from the Animal House of the Faculty of Pharmacy, University of Maiduguri, Borno State. They were housed in clean plastic, well-ventilated cages with saw dust as beddings under 12 hours light /12 hours dark cycle conditions of normal room temperature and humidity in the Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for the analysis. They were fed with standard fed (Vital Feed) and allowed water *ad libitum*.

Acute Toxicity (LD₅₀) Evaluation

The acute toxicity (LD₅₀) of *Beta vulgaris* crude methanol extract was determined using Lork's (1983) standard conventional procedure. In this study, two administration routes were considered: oral and intraperitoneal. In phase I, rats were divided into three groups of three rats each for each route (for a total of nine rats), and then treated with the crude ethanol extract at doses of 10, 100, and 1000 mg/kg bd. wt. intraperitoneally and orally for 24 hours and observed for mortality. In Phase II, each group (for each route) of animals was divided into three groups of one animal each, and the ethanol extract was administered at doses determined after Phase I. The rats were monitored daily for seven days for signs of toxicity and mortality during the first critical four hours. The LD₅₀ was then calculated using the formula:

kept constant by a 100watt bulb from an overhead lamp and were allowed 20 min to stabilize before starting the experiments. The initial systolic (SBP) and diastolic (DBP) blood pressures before addition of any drugs (basal blood pressures) were noted. The crude methanolic extract was dissolved in 50% dimethyl sulfoxide (DMSO) before administering to the rats. The effects of 50% DMSO alone followed by graded doses (50, 100 and 200 mg/kg) of crude methanol extract on the basal blood pressures were investigated.

The effects of a specific dose of the extract before and after administration of 1 mg/kg each of pentobarbitone were investigated. The hypertensive rats were divided into six groups of five rats each. Group 1 (Normal), Group 2 Hypertensive rats, while Group 3-5 were treatment groups and were administered doses of 50, 100 and 200 mg/kg respectively. Group 6 are positive control hypertensive rats and were administered 1 mg/kg of pentobarbitone. The SBP, DBP, MAP and HR were recorded.

Statistical Analysis

Results of pharmacological study were analysed using GraphPad Prism Version 8.0, 2016 Model for windows One-way Analyses of Variance (ANOVA) test followed by Tukey-Kramer's Multiple Comparison test was used to analyse and compare the results at 95 % confidence level. Values of $p < 0.05$ were considered significant. Results were expressed as mean \pm standard error of mean.

RESULTS

Phytochemical Constituents of the Crude and Column Fractions of Methanol Extract of *Beta vulgaris*

The results of phytochemical evaluation of the crude methanol root extract of *Beta vulgaris* revealed the presence of the major secondary metabolites such as carbohydrate, tannins, glycosides, cardiac glycosides, steroids, terpenes, and flavonoids with the exception of plobatannins, saponins and alkaloids.

Mineral Composition of the Root Extract of *Beta vulgaris*

The Results of the elemental analysis of *Beta vulgaris* root presented in the table 2 below revealed that, the concentration of sodium, lead, zinc, cadmium, iron, manganese nitrate, sulphate and phosphate of the elements analysed in relation to World Health Organization standard (WHO) safety threshold.

Proximate Contents of the Methanol Root Extract *Beta vulgaris*

The result of proximate analysis of the *Beta vulgaris* showed variation of percentage compositions of the nutrients. The proximate

content of the *Beta vulgaris* are presented in Table 3. The results revealed an increasing order of the nutrients as follows, Moisture content (82.72) > Dry matters (17.28) > Crude protein (6.25) > Crude fibre (5.82) > Carbohydrate (2.25) > Ash content (2.20) > Fat content (1.00).

Acute Toxicity (LD₅₀) Effects of the Methanol Root Extract of *Beta vulgaris* by Lorke's Method

The acute toxicity (LD₅₀) effect of the methanol root extract of *Beta vulgaris* which was determined using Lorke's method is shown in Table 4. The result revealed that, there was no mortality recorded in Phase I when extract doses of 10, 100 and 1000 mg/kg were administered. However, mortality was recorded at a dose of 5000 mg/kg. Thus the LD₅₀ was calculated as 3807.8 mg/kg.

The Blood Pressure of Experimental Animals before Administration of Crude Extract of *Beta vulgaris*

The blood pressure, Heart beat rate (HBR) and mean arteries pressure (MAP) of the experimental animals prior to the commencement of the experiment, the results indicate that all the experimental animals were normotensive. The result is presented in Table 5.

The Blood Pressure of the Experimental Animal after Induction with 1 mg/kg of pentobarbitone

The blood pressure of the experimental animals after induction with 1 mg/kg pentobarbitone revealed that all the animals are hypertensive with exception of group which served as normal control. This result of this study is shown in Table 6.

Effect of Methanol Crude Extract of *Beta vulgaris* on Hypertensive Rat (Day 1)

The result of blood pressure of the experimental animal after the administration of methanol crude extract with doses of 50, 100 and 200 mg/kg of *Beta vulgaris* on hypertensive rat on day one revealed progressive reduction on the blood pressure based on dose dependent with their percentage

reduction as 7.14%, 10.71%, 13.09% and 14.28% respectively. The result is presented in Table 7.

Effect of Methanol Crude Extract Root of *Beta vulgaris* on Hypertensive Rat (Day 3)

The result of the effect of methanol root crude extract of *B. vulgaris* on hypertensive rats on day three, revealed the percentage reductions of 9.36, 12.28, 16.37 and 18.17 at treatment doses of 50, 100 and 200. This study is shown in Table 8.

Effect of Methanol Crude Extract Root of *Beta vulgaris* on Hypertensive Rat (Day 5)

The effect of methanol crude extract root of *B. vulgaris* on hypertensive rat day five (5) is presented in Table 9. The result revealed that

at concentration of 200 mg/kg showed high reduction of the blood pressure of the experimental animals with percentage reduction of 22.28.

Effect of Methanol Crude Root Extract of *Beta vulgaris* on Hypertensive Rat (Day 7)

The result of the effect of methanol crude root extract of *B. vulgaris* on hypertensive rat at day seven (7) is shown in Table 10. The result revealed that the extract decrease blood pressure in dose dependent manner. It revealed that, there is a continuous progressive increase in % inhibition in hypertension induced in the Wistar rats. The most significant inhibition was observed at dose of 200 mg/kg with % reduction of 26.26.

Table 1: Phytochemicals constituent analysis of the crude methanol extract of *Beta vulgaris*

Test	Inference
1. Carbohydrates	+
2. Tannins	+
3. Phlobatannins	-
4. Glycosides	-
5. Cardiac glycosides	+
6. Steroids	+
7. Terpenes	+
8. Saponins	+
9. Flavonoids	+
10. Alkaloids	-

KEY: (+) Positive, (-) Negative

Table 2: Elemental and mineral composition of the root extract of *Beta vulgaris*

S/N	Element	Concentration (ppm)	WHO Standard (ppm)
1	Sodium (Na)	2.35	10-20
2	Lead (Pb)	0.18	0.2-20
3	Zinc (Zn)	0.09	15-20
4	Cadmium (Cd)	0.03	10-35
5	Iron (Fe)	0.08	0.5-50
6	Manganese (Mn)	1.37	200-1000
7	Nitrate (NO ₃ ²⁻)	4.90	5-6
8	Sulphate (SO ₄ ²⁻)	19.0	25-30
9	Phosphate (PO ₄ ²⁻)	4.20	21-35

Table 3: Proximate content of the methanol root extract *Beta vulgaris*

s/n	Content	Percentage (%)
1	Dry Matter	17.28
2	Moisture Content	82.72
3	Crude Protein	6.21
4	Crude Fibre	5.82
5	Ash	2.00
6	Carbohydrate	2.25
7	Fats (lipids)	1.00

Table 4: Acute toxicity (LD₅₀) effect of the methanol root extract of *Beta vulgaris* by using Lorke's method**PHASE I:**

Dose (mg/kg)	Mortality Rate
10	0/3
100	0/3
1000	0/3

PHASE II

Dose (mg/kg)	Mortality Rate
1600	0/1
2900	1/1
5000	1/1

Dead recorded at 5000 mg/kg. Thus the LD₅₀ is calculated as the square root of geometric mean dose of dead group x surviving group.

$$LD_{50} = \sqrt{a*b} = \sqrt{2900*5000} = 3807.8 \text{ mg/kg bwt}$$

Table 5: The blood pressure of experimental animals before administration of crude extract of *Beta vulgaris*

Group	Systolic (Mean±Sem) mmHg	Diastolic (Mean±Sem) mmHg	Heart Rate (Mean±Sem) mmHg	Beat	Mean Arterial Pressure (Mean±SEM) mmHg
Group 1	119.00±3.34	85.00±2.24	73.60±3.30		102.10±3.32
Group 2	118.00±3.33	86.00±1.87	75.20±1.85		102.20±3.33
Group 3	115.00±3.54	84.00±1.77	74.73±1,76		99.90±3.52
Group 4	115.00±1.72	86.00±2.51	76.20±1.70		100.70±1.87
Group 5	118.00±2.55	84.00±2.58	73.20±2.58		101.20±2.55
Group 6	119.00±1.63	85.00±2.48	74.00±2.46		102.50±1.62

N=5; N.T=Not treated

Table 6: The blood pressure of the experimental 1% pentobarbitone-induced Wistar strain rats

Group	Systolic (Mean±SEM) mmHg	Diastolic (Mean±SEM) mmHg	Heart Rate (Mean±SEM) mmHg	Beat	Mean Arterial Pressure mmHg
Normal	119.00±1.70 ^a	90.00±1.72 ^a	74.60±1.69 ^a		100.10±1.70 ^a
Induced N. T.	160.00±3.53 ^b	90.00±1.51 ^a	72.87±3.52 ^b		120.20±1.50 ^b
Group 3	153.00±4.63 ^c	88.00±1.20 ^b	67.33±3.99 ^c		121.50±1.19 ^b
Group 4	149.00±1.56 ^d	86.00±1.28 ^c	65.60±1.56 ^d		120.10±1.27 ^b
Group 5	151.00±2.48 ^e	86.00±2.11 ^c	68.40±2.47 ^e		120.90±2.10 ^b
Captopril	150.00±2.92 ^e	83.00±1.71 ^d	63.67±2.91 ^f		122.00±1.70 ^c

Key: n=5; Values along column with different superscripts are significantly different (p<0.05)

Table 7: Effect of methanol crude extract of *Beta vulgaris* on hypertensive rat Day 1

Group	Dose (mg/kg)	Systolic (Mean±SEM) mmHg	Diastolic (Mean±SEM) mmHg	Heart Beat Rate (Mean±SEM) mmHg	Mean Arterial pressure (Mean±SEM) mmHg
Normal		115.00±1.65 ^a	85.00±1.77 ^a	75.20±1.76 ^a	102.00±1.68 ^a
Induced N. T.	1mg/kg	168.00±3.31 ^b	90.00±1.06 ^b	64.06±1.02 ^b	129.00±3.33 ^b
Group 3	50 mg/kg	156.00±3.83 ^c (7.14)	86.00±1.70 ^a	62.40±1.68 ^c	121.40±3.88 ^c
Group 4	100 mg/kg	150.00±2.78 ^d (10.71)	83.00±1.59 ^c	61.53±1.60 ^c	121.40±2.67 ^c
Group 5	200 mg/kg	146.00±2.30 ^e (13.09)	85.00±1.51 ^a	64.66±1.49 ^b	115.50±2.34 ^d
Captopril	50 mg/kg	144.00±3.70 ^f (14.28)	84.00±1.56 ^c	60.40±1.30 ^d	115.50±3.72 ^d

Key: n=5; Values along column with different superscripts are significantly different (p<0.05); Values in the parenthesis show percentage reduction of Hypertension (HTN)

Table 8: Effects of methanol crude extract of *Beta vulgaris* on hypertensive rat Day 3

Group	Dose mg/kg	Systolic (Mean±SEM) mmHg	Diastolic (Mean±SEM) mmHg	Heart Beat Rate (Mean±SEM) mmHg	Mean Arterial Pressure (Mean±SEM) mmHg
Normal		115.00±1.88 ^a	86.00±1.70 ^a	76.20±1.69 ^a	100.70±1.87
Induced N.T	1 mg/kg	171.00±4.47 ^b	86.00±1.43 ^a	58.00±1.42 ^b	129.00±3.98
Group 3	50 mg/kg	155.00±3.51 ^c (9.36)	83.00±1.85 ^b	60.00±3.30 ^c	119.50±3.50
Group 4	100 mg/kg	150.00±3.38 ^d (12.28)	84.00±1.51 ^b	61.93±1.54 ^c	117.10±3.36
Group 5	200 mg/kg	143.00±2.15 ^e (16.37)	82.00±1.86 ^c	61.93±1.54 ^c	114.30±2.14
Captopril	50 mg/kg	139.00±3.43 ^f (18.17)	82.00±1.24 ^c	65.26±1.22 ^d	121.60±3.40

Key: n=5; Values along column with different superscripts are significantly different (p<0.05); ()= Values in the parenthesis show percentage reduction of Hypertension (HTN); N.T= Not Treated

Table 9: Effect of crude methanol extract root of *Beta vulgaris* on hypertensive rat Day 5

Group	Dose (mg/Kg)	Systolic (Mean±SEM) mmHg	Diastolic (Mean±SEM) mmHg	Heart Beat Rate (Mean±SEM) mmHg	Mean Arterial Pressure (Mean±SEM) mmHg
Normal		115.00±1.32 ^a	85.00±1.86 ^a	75.40±1.77 ^a	100.40±1.33 ^a
Induced N.T	1 mg/kg	175.00±3.86 ^b	53.93±1.42 ^b	53.93±1.42 ^b	129.60±3.83 ^b
Group 3	50 mg/kg	151.00±2.61 ^c (13.71)	84.00±1.46 ^a	62.52±1.37 ^c	118.20±2.60 ^c
Group 4	100 mg/kg	143.00±3.05 ^d (18.28)	81.00±1.46 ^c	61.33±1.45 ^d	125.50±2.50 ^d
Group 5	200 mg/kg	136.00±2.56 ^e (22.28)	85.00±1.50 ^a	68.33±1.49 ^e	111.00±2.54 ^e
Captopril	50 mg/kg	137.00±3.32 ^e (21.71)	84.00±2.05 ^a	59.33±2.00 ^f	117.00±3.30 ^c

N=5; Values along column with different superscripts are significantly different (p<0.05); (P<0.05); ()= Values in the parenthesis showed percentage reduction of Hypertension (HTN)

Table 10: Effect of methanol crude root extract of *Beta vulgaris* on hypertensive rat Day 7

Group	Dose mg/kg	Systolic (Mean±Sem) mmHg	Diastolic (Mean±SEM) mmHg	Heart Beat Rate (Mean±SEM) mmHg	Mean Arterial Pressure mmHg
Normal		119.00±2.00 ^a	85.00±1.15 ^a	71.65±2.11 ^a	98.60±1.11 ^a
Induced N. T.	1 mg/kg	179.00±3.59 ^b	88.00±1.43 ^b	57.93±1.42 ^b	134.10±1.40 ^b
Group 3	50 mg/kg	149.00±3.59 ^c (13.71)	88.00±1.43 ^b	57.93±3.56 ^b	118.20±2.97 ^c
Group 4	100 mg/kg	137.00±2.79 ^d (23.46)	87.00±1.68 ^b	68.73±1.22 ^c	111.40±2.70 ^d
Group 5	200 mg/kg	132.00±2.44 ^e (26.26)	85.00±1.65 ^a	70.20±1.67 ^d	109.00±2.44 ^e
Captopril	50 mg/kg	137.00±3.45 ^d (23.46)	86.00±1.20 ^a	67.20±2.95 ^c	117.90±3.41 ^c

Key: n=5; Values along column with different superscripts are significantly different (p<0.05); ()= Values in the parenthesis show percentage reduction of Hypertension (HTN)

DISCUSSION

The results of phytochemical constituents present in both the crude methanol extract of *Beta vulgaris* revealed the presence of alkaloid, Flavonoids, terpenoids, cardiac glycosides, phenols, tannins, steroids, and carbohydrates but devoid of saponins and anthraquinone. Plants are rich bioresources of drugs and a few interesting outcomes have

been found with the use of a mixture of natural products or plants extracts to treat diseases (Gibbon, 2003). Flavonoids have been reported to have antihypertensive activities in the laboratory animals. Example flavonoids from *Hippophae rhamnoides* (Zhu *et al.*, 2005) and *Astragalus complatus* (Xue *et al.*, 2002) also reduce blood pressure. Furthermore, flavonoids have been widely described in the

literature as vasodilator compounds (Duarte *et al.*, 1999; Fitzpatrick *et al.*, 1993; Herrera *et al.*, 1996). Terpenoids is a heart friendly phytochemical constituent and this may be the reasons why *Beta vulgaris* is very popular among patients with high blood pressure and diabetes because it helps to reduced diastolic blood pressure and lower sugar level in blood (Hawkins and Ehrlich, 2006). From clinical studies, it is shown that terpenoids strengthen the skin, increase the concentration of oxidants in wounds, and restore inflamed tissues by increasing blood supply. Because of these properties *Beta vulgaris* has been used in herbal medicine for burns, prevent scar formation following surgery, from an episiotomy following vaginal delivery of a newborn and treatment of fistulas (Hawkins and Ehrlich, 2006). Cardiac glycosides were found to be present in *Beta vulgaris*, a compound that has been shown to aid in treatment for congestive heart failure and cardiac arrhythmia. This is another reason why this plant is widely used in traditional medicine. Cardiac glycosides work by inhibiting the Na⁺ pump. This causes an increase in the level of sodium ion in the myocytes, which then leads to a rise in the level of calcium ions available for the contraction of the heart muscle, improves cardiac output and reduces distention of the heart. The results of the present study showed the presence of tannin which was reported to lower blood pressure in renal hypertension; this is similar to the action of green tea tannin. Renal hypertensive rats given green tea tannin showed a significant decrease in the systolic, mean, and diastolic blood pressure values. The administration of green tea tannin increases in the extraction the low urinary kallikrein level, with increase in the excretion of sodium (Na). Similarly, the results of the proximate composition of *Beta vulgaris* revealed that the moisture content value of *Beta vulgaris* revealed high value of 82.72%. This high moisture value is easily prone to deterioration (Kwenin, 2011). The high moisture content

may induce a greater activity of water-soluble enzymes and co-enzymes involved in metabolic activities of the vegetables which include *Beta vulgaris* (Iheanacho and Udebuani, 2009). Likewise, the crude fibres content of *Beta vulgaris* revealed the percentage of (15.00 %) when compared to the values (22.33 %) by FAO (2006). The crude fibre would be advantageous for their active role in the regulation of intestinal transit, increasing dietary bulk due to their ability to absorb water (Jenkin, *et al.*, 2017). Adequate intake of dietary fibre can lower serum cholesterol level, risk of coronary disease hypertension, constipation, diabetes, and breast cancer It is necessary for health condition, curing of nutritional disorders and for food digestion (Sizer and Whitney, 2003). The crude protein content of *Beta vulgaris* showed 6.21 % lower percentages as compared to other vegetable such as *V. unguiculata* (21.96), *Momordica balsamina* (11.29%) [Ashaolu *et al.*, 2012]. It's worth précising that plant foods which provide more than 12% of their calorific value from protein have been shown to be alternative sources of protein (Ali, 2009). This suggests that *Beta Vulgaris* are good sources of proteins and could play a significant role in providing cheap and available proteins for rural communities. In addition, protein in form of amino acid are nutrients needed by human body for growth and maintenance (Nelson and Cox, 2005; Lal, 2008; Garrett and Grisham, 2005). Protein is needed to form blood cell, protects, rebuilds, maintains and grows tissues, skin hair, muscle, connective tissue, bone marrow and vital organs (Lal, 2008). Carbohydrates supply energy for the nutrition, and they are readily fermented by microorganisms to yield carbon dioxide, alcohol, organic acids and other compounds (Sizer and Whitney, 2003). The result of this study showed that, the percentage of carbohydrate is lower (2.25%) compared to other vegetable such as *Ocimum gratissimum* (4.72%).

The crude lipids content revealed 1.00% showing the lowest content, these can be compared with some of the vegetable's fats content (8.3%) as reported by Fagbohun *et al.* (2012). This shows that *Beta vulgaris* are poor source of lipids. However, it's important to note that diet providing 1-2% of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption yield to cardiovascular disorders such as atherosclerosis, cancer, and aging (Kris-Etherton *et al.*, 2002).

The elemental analysis results indicate that the concentration of some of the trace essential elements such as Manganese (Mn), Sodium (Na), Zinc (Zn), lead (Pb), Cadmium (Cd), Iron (Fe), Nitrate (NO_3^{2-}), Sulphate (SO_4^{2-}) and Phosphate (PO_4^{2-}) analysed in *Beta vulgaris* are all within the safety limits as reported by World Health Organization (WHO, 1996) as shown in the Table 4.3. The highly soluble mineral elements: sodium (Na), Magnesium (Mg), phosphorus (P), Iron (Fe) help in the maintenance of acid-base balance of hydrogen ion concentration of the body (Vasudevan, 2005). They help to complete the absorption of vitamins, proteins, fat, and carbohydrate of food. Sodium (Na) and Iron (Fe) furnish all the cells and tissues of the body with the elements and the nutritional enzymes which they need. According to Geissler and Powers (2005), iron plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important catalytic Centre in many enzymes as the cytochromic oxidase. *Beta vulgaris* could be recommended in diets for reducing anemia hypertension which affects more than one billion people worldwide (Trowbridge and Martorell, 2002). Calcium with phosphorus is required for the formation of the bone and teeth and in the proper functioning of the nervous system.

Magnesium (Mg) is needed for acid base and electrolyte balance in the body. Since magnesium is useful in the reduction of blood pressure, magnesium is an obligate co-factor of DNA synthesis and the proportion supplied

by green leafy vegetables can be used to supplement low magnesium based staple foods such as cassava. Magnesium deficiency may play a major role in some cases of anaemia. Deficiency of Magnesium has been shown to produce spasms of the coronary arteries and is thought to be a cause of non-exclusive heart attacks (Kirschmann, 1979).

An adequate intake of zinc enhances the ability to promote healthy skin and hair. It also improves reproductive function (Nelson and Cox, 2005). Manganese is essential for the proper formation and maintenance of bone, cartilage, and connective tissue, it contributes to the synthesis of proteins and genetic material and helps produce energy from foods. Accordingly, level contributed by the organic matter applied to the farm has not reached the nuisance level. Consumption of *Beta vulgaris* with reported phosphate concentration aid in the formation of bones and teeth, kidney functioning muscles contraction, normal heart beat and nerve signaling in human (35). The presence of varying concentrations of heavy and trace elements in the *Beta vulgaris* may be a reflection of the amount in the soil which may be due to the topography, soil water plant exchange complex and evaporation or evapotranspiration of the environment (Adoun *et al.*, 1998; Abdulrahman, 2004)

The results of the toxicity study of *Beta vulgaris* (LD_{50}) in rats was determined to be 1450mg/kg. On administration of 10 mg/kg, 100 mg/kg, and 1000 mg/kg doses of the extract via oral route, there was no death recorded which makes the LD_{50} of the methanol root extract via oral route to be greater than five thousand (≥ 500 mg/kg). Clinical signs observed were loss of appetite, difficulty in breathing, transient dullness, weakness and paralysis of limbs and these early symptoms subsequently disappeared after few minutes. According to toxicity class of Hodges (2005), any compound with $\text{LD}_{50} \geq 5000$ mg/kg should be considered safe or harmless and thus, administration as done in

folk medicine may not have any immediate adverse effects.

The effects of methanol root extract of *Beta vulgaris* on systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) in hypertensive rats. Baseline, there was no significant difference in SBP, DBP, MAP and HR among the experimental group. Treatment with this extract of *Beta vulgaris* reduced ($P < 0.05$) systolic, diastolic, and mean arterial in both normotensive and hypertensive rats compare to the vehicle group. At the doses of 50 mg/kg, 100 mg/kg, and 1000 mg/kg of the oral administration of the crude extract of *Beta vulgaris* reduced the basal mean arterial pressure of normotensive rats.

From day one, three, five, the results of the experiment with the crude extract are as follows. 136 ± 3.30 mmHg systolic, 85 ± 1.61 mmHg diastolic, and 111.0 ± 2.54 mmHg mean arterial pressure. The blood pressure lowering effect of the crude extract was not affected by 1 mg/kg of pentobarbitone. It is noteworthy that methanol root extract of *Beta vulgaris* produced a significant reduction in blood pressure and heart rate of hypertensive rats. The results suggest that the extract has antioxidant effect and may contribute to blood pressure reduction. The antioxidant effect of

the extract is due to its strong scavenging effect on reactive oxygen species and free radical as reported by Chen *et al.* (2013). The preliminary phytochemical screening on methanol root extract detected the presence of flavonoids, tannin, terpenoids, steroids and phenol. Flavonoids are among the antihypertensive materials for antihypertensive efficacy has been extensively confirmed (Cassidy *et al.*, 2011). In view, of the hypertension model used, active secondary metabolites detected in *Beta vulgaris* could either act singly or in synergy with one another.

CONCLUSION

The results of both the proximate and mineral elements analysed using standard protocols revealed that the plant is nutritive and the parameters were within the threshold level set by World Health Organization. The plant contains phytochemicals which include flavonoids, Terpenoids, tannins, steroids and cardiac glycosides. The acute toxicity (LD_{50}) study revealed that the calculated median lethal dose is 3807.8mg/kg The methanol root extract at 200mg/kg dose was observed to have highest % reduction of hypertension compared to the standard drug (Captopril, 50mg) used for this study

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