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Research Article

Antinutritional and Antibacterial Activity of Aqueous and Ethanolic Extracts of *Vernonia amygdalina* (Bitter Leaf)

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Abstract

Human being has used plants to treat common infectious disease and some of these traditional practices are still included as part of the habitual treatment of various maladies. Today, researches are focusing on the discovery of new therapeutic substances of natural origin with possible low or no toxicity to humans, animals and the environment based on the ethnomedicinal. Despite the fact that the leaves of *V. amygdalina* are widely consumed because of the accepted nutritional values. However, antinutritional composition and antibacterial activity present in bitter leaf has not been studied. Therefore the present study was conducted to fill these gap. Hence, the study was initiated to determine antinutritional composition and antibacterial activity of aqueous and ethanolic extracts of *Vernonia amygdalina* (bitter leaf). Aqueous and ethanolic extracts of leaves of *Vernonia amygdalina* a member of Asteraceae family, were used to investigate their antibacterial effects against pure cultures of clinical isolates of *Staphylococcus aureus, Ecsherichia coli, Pseudomonas aeruginosa and Proteus vulgaris.* Graded concentrations of 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml of both water and ethanolic extracts where used as the stock solution of the plant extract. The presence of anti-nutritional factors: tannins, oxalate and phytic acid were determined. From the results obtained, *V. amygdalina* leaf extracts proved effective against the test organisms with ethanolic extract having the highest effects as it showed wider zones of inhibition to the test organisms at 800mg/ml and 400mg/ml; with a minimum inhibitory concentration and an minimum bactericidal concentration at 800mg/ml. The water extract was also effective against *Ecsherichia coli* and *Pseudomonas aeruginosa*, with narrower zones of inhibition at 800mg/ml and 400mg/ml with a minimum inhibitory concentration at 1600mg/ml. The results also revealed that the *V. amygdalina* leaf contains anti-nutritional factors with 48.14 mg/100g for phytic acid, 237.67 mg/100g for oxalate and 3.43 mg/100g for tannin. This research has shown that *V. amygdalina* leaves have great antibacterial activity, but they also contain some amount of antinutrients which can alter the nutritional content of the plant in humans and animals.

Keywords: Antinutritional Factors; Antibacterial Activity; Bitter Leaf

Introduction

Green leafy vegetables are important components of the dietary regime of humans because they provide necessary vitamins and minerals [1]. However, they also contain antinutritional factors which reduce the bioavailability of these nutrients [2]. Aletor and Adeogun [3] reported that some antinutritional factors exhibit protective effects, thus making them serve dual purpose. Notable examples of these antinutritional factors include; oxalate, phytates, tannins, saponins, protease inhibitors, cyanogenic glycosides and so on.

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potentials said contained what we currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious disease and some of these traditional are still included as part of the habitual treatment of various maladies [4]. Today, researches are focusing on the discovery of new therapeutic substances of natural origin with possible low or no toxicity to humans, animals and the environment based on the ethnomedicinal and ethnoveterinary practices [5]. *Vernonia amygdalina,* a member of Asteraceae family, commonly known as "bitter leaf" because of

its bitter tasting leaves, is a rapidly generating short, wooded shrub that grows up to 2-6 meters high in African tropics and other parts of the world. The potentials of *V. amygdalina* was first noted when scientists observed chimpanzees use the pitch of the shrub for self disparasitilization [6]. Since the discovery, subsequent researches has unveiled more and more bioactivities possessed by different extracts of this plant such as; antidiabetic, antimalarial, antifungal, antioxidant, cytotoxic effects and so on which are beneficial to health [5]. Compounds including steroid glycosides, sesquiterpene lectone and flavonoids contribute to the bitter taste of this plant [7].

V. amygdalina a member of Asteracea family is a small shrub that grows in tropical Africa with leaves of about 6 mm in diameter, elliptical in shape, veined and bears pale hairs on the underside. *V. amygdalina* typically grows to a height of about 2-6 meters. It has grey or dark bark that has a rough texture; the branches of the shrub are brittle and break off easily. It bears whitish small, clustered flowers. It has a bitter taste and a characteristic odour [8]. No seeds are produced therefore; the plant is distributed through cutting. It grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant [9]. In Nigeria, and some other African countries, *V. amygdalina* is known by various names. Table 1 shows the different names of *V. amygdalina* in various countries.

Despite the fact that the leaves of *V. amygdalina* are widely consumed because of the accepted nutritional values, there is lack of sufficient information on the antinutritional factors present in the plant. This research was therefore undertaken to determine both the levels of some of the antinutritional factors present in *V. amygdalina* and the antibacterial effects of its leaf extracts on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris*.

Materials and Methods

Collection of plant materials

Fresh leaves of *Vernonia amygdalina* (bitter leaf) were collected at Kufang District, Jos (Latitude 09°55'00'' N and Longitude 08°53'25'' E) Plateau state, Nigeria. The fresh leaves where collected and rinsed with distilled water to remove the dirt. They were dried at room temperature and then pulverized into fine powder using mortar and pestle and stored in an air tight container prior to analysis.

Plant extracts preparation

- **Cold Water Extract:** Fifty grammes (50g) of each pulverized sample was weighed and transferred into a conical flask and 500ml of de-ionized water was dispensed into it. Cold water extraction was carried out so as not to denature or damage plant constituents. The mixture was agitated vigorously to ensure proper mixing, after which it was allowed to stand for 24hours. The mixture was then filtered using a Whatman № 1 filter paper. The filtrate was then concentrated using a rotatory evaporator (Shanghai Shen Sheng Biotech Co. Ltd., China) fitted with a vacuum pump. The resultant extract was labelled and stored in a clean, air-tight sample bottle at room temperature until analysis.
- **Ethanolic Extract:** Fifty (50g) of each pulverized sample was weighed on an analytical weighing machine (Ohaus Corp., USA) To this sample, 500ml of absolute ethanol (inorganic solvent) was added in a conical flask. The flask was tightly covered by muffling cotton wool into the tip which was made firm with masking tape to prevent evaporation. The mixture was then agitated carefully for 2-3minutes and allowed to stand for 72hours, after which it was filtered using a Whatman № 1 filter paper. The filtrate was evaporated to dryness using an oven (Gallenkhamp 300 series) at a temperature of 55°C and then placed into clean labelled sample bottle and stored at room temperature.

Bacteriological study

Flavour bottles, pipettes, and test tubes were washed with detergent and a reasonable amount of hypochloric acid (bleaching agent). After which they were properly rinsed with distilled water and dried at 160^0C in the oven for 1 hour. The cork borer was disinfected using alcohol. This was followed by a thorough rinsing with sterile distilled water.

Mueller-Hinton agar (38g) was suspended in 1 litre of distilled water in a conical flask. It was then brought to boil to dissolve the medium completely. Sterilization was done by autoclaving (Memmert, Germany) at 121°C for 15minutes.

Media composition

beef, dehydrated infusion 300.0, casein hydrolysate 17.5, starch 1.5, agar 17.0

Preparation of stock solution of plant extracts

From the cold water extract, graded concentrations of 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml were measured and separately dispensed into flavour bottles and each

was diluted with 1ml of sterile distilled water to obtain five (5) different concentrations of cold water extract (i.e. 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml). These where then labelled and stored at 8°C-12°C to be used for the susceptibility test, minimum inhibitory and minimum bactericidal concentration determinations.

For the ethanolic extract, graded concentrations of 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml of the extract was measured and separately dispensed into flavour bottles and each was distilled with 1ml of dimethylsulfoxide (DMSO) to obtain 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml concentrations. This where then stored at 8°C-12°C to be used for the susceptibility test, minimum inhibitory and minimum bactericidal concentration determinations.

Preparation of test bacterial isolates

Clinical isolates of multi-drug resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa, E. coli,* and *Proteus vulgaris* obtained from the Bacteriology Department of Federal College of Medical Laboratory Technology (FCVMLT) Vom, Nigeria, were made from the fresh plates of test bacterial culture obtained in Stocks, and were maintained in agar slants. Colonies of fresh cultures of the different bacterial isolates were picked and suspended in 5ml Mueller-Hinton broth in well-labelled sterile Bijou bottles for incubation at 37^{0}C prior to antimicrobial testing of each.

Antibacterial activity test

The cold water and the ethanolic extracts were then subjected to antimicrobial study to determine the susceptibility, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extracts against bacterial isolates.

Antibacterial sensitivity (Agar Well Diffusion Assay)

The susceptibility test of the microorganism to the leaf extract was done according to the agar-well diffusion method described by [10]. The bacterial suspension was inoculated in Mueller-Hinton agar plates using the spread plate method [10] so as to achieve even growth on the plates and incubated at 37°C for 30 minutes. Wells were then bored into the agar at equidistance using a sterile cork borer. The wells were then carefully filled with 0.1ml each of the various extracts concentrations (800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml). Ciprofloxacin (0.625mg/ml) was used as positive control while water served as negative control. The plates where then incubated at 37°C for 24 hours. The relative susceptibility of the organisms to the plant extract was demonstrated by a clear zone of inhibition around the wells containing the extracts of different concentrations. The plates were observed for zones of inhibition macroscopically (with unaided eyes). These zones of inhibition were then measured to the nearest whole millimeter (mm) using a clear transparent ruler. If no growth was observed, it means the extract has an inhibitory effect on the test organism at that concentration, but if growth occurs, then the extract has no inhibitory effect on the organism.

Minimum inhibitory concentration (MIC)

This was done to determine the lowest concentration of the extract that will inhibit the visible growth of test organism. This was carried out on those concentrations that showed inhibitory effect(s) on the test microorganisms. Then, doubling dilution method was employed from Abdullahi., *et al*. [11] with slight modifications. Five (5) test tubes were used to determine the MIC of each extract that showed inhibitory effect at a given concentration tested. To each of the 4 test tubes, 9ml of Mueller-Hinton broth containing the suspended organism was added following the addition of one millilitre (ml) of the leaf extracts. The content of the test tubes where mixed thoroughly, 1ml of the first test tube content was then transferred to the second test tube and mixed properly, 1ml of the second test tube content was also transferred to the third test tube and mixed properly, this continued until the last test tube (tube 5) was reached. From tube 5, 1ml of its content was also discarded. This process was repeated for all the extract concentrations that showed inhibitory activity. The tubes where incubated at 37°C for 24 hours after which the tubes were macroscopically examined for the presence of turbidity. The tubes that showed turbidity were tubes that had microbial growth while those that showed no turbidity where tubes that had no microbial growth. The lowest concentration of the extract that showed no turbidity (inhibited the growth of the test bacteria) was taken as the MIC.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBCs) is the lowest concentration of the extract that will prevent the growth of test organisms after subculture on to antibiotic-free media. Minimum bactericidal concentration of the plant extract was determined according to the method of Igbinosa., *et al*. [10]. Those dilutions that showed no turbidity for the Minimum Inhibitory Concentration (MIC) assay were sub-cultured on Mueller-Hinton agar plates. A loopful from each of these tubes were sub-cultured and incubated at 37°C for 24 hours and examined for the presence or absence of growth. The lowest concentrations that still showed no growth were noted as the Minimum Bactericidal Concentration (MBC) for each of the extract.

Antinutrients analysis of the plant

Determination of phytic acid

Two grammes of the sample was weighed and dissolved in a 100ml beaker. Fifty ml of 0.5N HCL was then added. The solution was mixed properly and left to stand overnight. Using a whatman No.1 filter paper, the mixture was filtered, and 12 ml of the filtrate was dispensed in a 100 ml beaker, and neutralized with 12 ml of 10 N NaOH then mixed properly. Two drops (1ml) of 20% ferric chloride ($FeCl_2$) was added to precipitate the phytates in the sample. The solution was heated for 15 minutes to convert phytates to ferric phytates). This was allowed to cool and then centrifuged at 7000 rpm for 7 minutes. The supernatant was discarded and 3 ml of 0.17 N HCl was added so as to remove the deposit from the centrifuge tube. The remaining deposit was rinsed with 5mls of hot distilled water into the 100 ml beaker and heated for 15 min. Three ml of 0.5N NaOH was added and further heated for 15mins. This was allowed to cool and again, centrifuged again at 7000 rpm for 7 minutes. The supernatant was discarded and rinsed with 5 ml of hot distilled water. One ml of concentrated H_2SO_4 and 1 ml of perchloric acid where added. It was heated on a digestion heater under fume cupboard until dried yellow cake is obtained. It was removed and allowed to cool. To this, 20mls of distilled water was added to dissolve it. The pH was adjusted to 7.0 (using 10 N NaOH and 6 M HCl). This was then filtered and the volume made up to 50 ml with distilled water. In a clean test tube, 1 ml of the sample was dispensed and in another, 1 ml of distilled water which serves as blank. To both, 3 ml of copper acetate buffer was added then 0.5 ml of 5% ammonium molybdate and metol (freshly prepared) were also added, allowed to stand for 5 minutes and read in a spectrophotometer at 470 nm; calibrated with blank.

Calculation:

Using K×A×9.92

where;

K = average reading of standards

A = absorbance of sample

9.92 = is a factor

Determination of oxalate

Two grammes of the powdered sample was weighed and put in 250ml conical flask, 10 ml of 6 N HCl was added. To this, 190 ml of distilled water was also added, mixed properly and allowed to stand overnight. The mixture was filtered and was made up to 250 ml. Fifty ml of the filtrate was measured out in 100ml beaker, 2-3 drops of methyl red indicator was added followed by concentrated ammonia dropwise until a faint yellow colour was obtained. This was then heated on a hot plate until it boiled. It was removed and allowed to cool, then filtered. Heated again until it boiling. Then, 10 ml of 5% $CaCl_2$ was added. Further 5 ml of 5% $CaCl_2$ was added then heated for 5mins. It was removed and allowed to stand overnight in a fridge and observed for precipitates. It was filtered using a Whatman №.1 filter paper, the precipitate was washed into a beaker using 5ml of 1:4 sulfuric acid (25%). The filter paper was rinsed with 5ml of Hot distilled water into the beaker and placed on hot plate to boil. It was titrated using 0.5N potassium permanganates while hot. It was also titrated on blank using 5ml (3ml 1:4 H_2SO_4 and 2ml distilled water).

Calculation

Titre value of blank subtracted from that of sample, multiplied by 50 = oxalate in mg/100g of sample

Determination of tannins

Five gramme of sample was weighed in a 250 ml conical flask. On to this, 250 ml of hot distilled water was added. This was allowed to stand for about 3 hours and then filtered. The filtrate was allowed to evaporated in an oven at 60°C till it dried. The dried sample was dissolved in 20 ml of distilled water, filtered, and made up to 25 ml with distilled water. After the dilutions, it was allowed to stand for 50 minutes, and then read at 760 nm in a spectrophotometer.

Calculation

Tannins mg/100g sample= C×25×20

where;

C = Conc. due to 1ml of extract, extrapolated from standard curve

25 = sample dilution value

20 = conversion factor (100/5) to 100g

Statistical analysis

The anti-nutrient compositions were determined by different methods, and data obtained were subjected to analysis of variance to determine any significant difference at 5% level ($p<0.05$) using SPSS version 17.0 and was reported as means of three replicates.

Results and Discussion

Results

The tables below present the respective antimicrobial examination of the plant sample

The antinutritional analysis where carried out on the leaves of *Vernonia amygdalina* and the factors analysed for include; phytic acid, oxalate and tannins. The results are as follows;

Test Organism	Zones of Inhibition (mm)					
	Conc. of Aqueous and Ethanolic Extract (mg/ml)					
	800 A E	400 A E	200 A E	100 A E	50 A E	Cif (0.625) A E
Staphylococus aureus	- 20	- 17	- -	- -	- -	28 30
Escherichia coli	10 20	7 18	- -	- -	- -	27 28
Pseudomons aeruginosa	12 22	- -	- -	- -	- -	25 27
Proteus vulgaris	- 15	- -	- -	- -	- -	25 27

Table 1: Susceptibility of the test bacteria to aqueous and ethanolic extract of the plant sample.

KEY

- = No zone of inhibition, Cif = Ciprofloxacin standard drug (positive control), A = Aqueous Extract, E = Ethanolic Extract

Test Organism	Minimum Inhibitory Concentration							
	Conc. of Aqueous and ethanolic extract (mg/ml)							
	1600 A E	800 A E	400 A E	200 A E	100 A E	50 A E	MIC A E	
Staphylococcus aureus	- -	+ -	+ +	+ +	+ +	+ +	1600	800
Escherichia coli	- -	+ -	+ +	+ +	+ +	+ +	1600	800
Pseudomonas aeruginosa	- -	+ -	+ +	+ +	+ +	+ +	1600	800
Proteus vulgaris	- -	+ -	+ +	+ +	+ +	+ +	1600	800

Table 2: The minimal Inhibitory Concentration (MIC) of Aqueous and Ethanolic leaf extract of *Vernonia amygdalina.*

KEY

- = No Growth, + = Growth, A = Aqueous Extract, E = Ethanolic Extract

Test Organism	Minimum Bactericidal Concentration						
	Conc. of Aqueous and Ethanolic Extract (mg/ml)						
	1600 A E	800 A E	400 A E	200 A E	100 A E	50 A E	MBC A E
Staphylococcus aureus	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Escherichia coli	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Pseudomonas aeruginosa	+ -	- +	+ +	+ +	+ +	+ +	+ 800
Proteus vulgaris	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Table 3: Minimal Bactericidal Concentration (MBC) of Aqueous and Ethanolic leaf Extract of Vernonia amygdalina.

KEY

- = No Growth, + = Growth. A = Aqueous Extract, E = Ethanolic Extract

Table 4 shows results obtained for anti-nutritional factors analysis of *Vernonia amygdalina.* From the result, it can be seen that *V. amygdalina* has a phytic acid content of 48.14 ± 0.18, an oxalate content of 237.67 ± 2.57 and a tannin content of 3.43 ± 0.53.

Sample	Antinutritient (mg/100g)		
	Phytic acid	Oxalate	Tannins
Vernonia amygdalina	48.14±0.18	237.67±2.57	3.43±0.53

Table 4: Mean levels of some antinutritional factors in V. amygdalina

*Tabulated values are mean ± standard deviation of 3 determinations per sample.

Discussion

Vernonia amygdalina leaf has been found to contain natural antibacterial agents that are effective against common bacteria. Four (4) common bacteria were used in this research work; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris.* Both extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial species (Table 3).The results of the sensitivity test showed that both the aqueous and ethanolic extracts showed zones of inhibition to the highest concentrations used (800mg/ml and 400mg/ml). The ethanolic extracts had wider zones of inhibition; the result revealed that the extract obtained with ethanol solvent showed more antimicrobial activity to the test organism than that of water solvent. This might be explained by the ability of ethanol to dissolve multivariable types of compounds; polar and non-polar, simple and complex chemical structures compared with water, this is presented in Table 3. The results obtained showed that activities of the extracts increased with increasing concentration, the test organisms where more susceptible to the ciprofloxacin (0.625mg/ml) with zones of inhibition ranging from 25-30mm compared to both plant extracts. The susceptibility test results showed *Staphylococcus aureus* inhibitory diameter for the ethanolic extract where; 20mm and 17mm at 800mg/ml and 400mg/ml respectively, no zones of inhibition where observed for the aqueous extract. *Escherichia coli* inhibitory diameter for ethanolic extract where 20mm and 18mm at 800mg/ml and 400mg/ml respectively while that of aqueous extract was 10mm and 7mm for 800 and 400mg/ml respectively. *Pseudomonas aeruginosa* had 22mm for ethanolic extract and 12mm for aqueous extract at 800mg/ml. *Proteus vulgaris* had 20mm zone of inhibition foe ethanolic extract at 800mg/ml. The test organisms where all susceptible to the crude extracts since they all had ≥7mm inhibition zones at their highest concentrations 800mg/ml [14].

The MIC results in Table 4 showed that the lowest concentration at which the extracts can inhibit the test organisms is 1600mg/ml for aqueous extract and 800mg/ml for ethanolic extract. The MBC results in Table 5 showed that 800mg/ml of the ethanolic extract is the lowest concentration at which the extract can totally kill *Pseudomonas aeruginosa*.

The findings show the effectiveness of ethanolic extracts of *V. amygdalina* than aqueous extract of the same plant due to its better extraction power as organic solvent. Eloff [15] also reported that most active component of plants are not water soluble. The high activity of ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists [16]. The ethanonic extracts of *V. amygdalina* showed a better antibacterial activity than aqueous extract but not comparable to the standard antibiotic (ciprofloxacin) used. The potency of ethanoloic extract to the organisms was about three to four times higher than that of aqueous extract.

The anti-nutritional analysis revealed that although there are lots of advantageous constituents attributed to *V. amygdalina*, they also contain some amount of toxic substance (anti-nutritional factors) some of which were assayed; Phytic acid which was 48.14 ± 0.18(mg/100g), Oxalic acid which was 237 ± 2.27(mg/100g) and Tannins which was 3.43 ± 0.53(mg/100g). Antinutritive factors limit the use of many plants due to their ubiquitous occurrence as natural compounds capable of eliciting deleterious effect in man and animals [17]. These anti-nutritional factors to an extent reduce the bioavailability of the nutrients present in the plant. Oxalate tends to render calcium unavailable by binding to the calcium ion to form complexes [18,19]. The insoluble calcium oxalate may precipitate around soft tissues like kidney, causing kidney stones [20,21]. The value obtained from this study is considered below toxic level. Phytic acid acts as a strong chelator forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability [22,23]. Phytate is associated with nutritional diseases such as rickets and osteomalacia in children and adult, respectively. According to Oke [21] a phytate diet of 1-6% over a long period of time decreases the bioavailability of mineral elements in mono gastric animals. This value obtained is below toxic level. Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur in all vascular plants. Tannin binds to proteins making them biounavailable [24-26]. These values are low compared with those of other workers [27-31]. Finally, the results indicated the presence of antinutritional factors but they were present in low levels that there is no fear of significant intervention with nutrients utilization.

Conclusion

The antimicrobial activity of the leaf of *V. amygdalina* on bacteria of the test isolates have been relatively established in this study and could possibly justify the claims by the traditional healers in its use to treat infectious diseases. The result of this research has shown that *V. amygdalina* leaves have great antibacterial activity and some bioactive compounds but they also contain some amount of antinutrients which can alter the nutritional content of the plant in humans and animals. Further studies are needed to determine and also characterize the phytochemicals responsible for these activities in *V. amygdalina*.

Bibliography

1. Fasuyi AO. "Nutritional potentials of some tropical vegetable meals. Chemical characterization and functional properties". *African Journal of Biotechnology* 5.1 (2006): 49-53.

2. Akindahunsi AA and Salawu SO. "Phytochemical screening and nutrient and anti-nutrient composition of selected tropical green leafy vegetables". *African Journal of Biotechnology* 4 (2005): 497-501.

3. Aletor VA and OA Adeogun. "Nutrient and anti-nutrient components of some tropical leafy vegetables". *Food Chemistry* 53 (1995): 375-379.

4. Heinrich M., *et al*. "Fundamental of pharmacognosy and phytotherapy". Curchill Livingstone, Edinburgh. 2004.

5. Swee KY., *et al*. "Vernonia amygdalina, an ethnoveterinary and ethnomedicinal used green vegitable with multiple bioactivities". *Journal of Medicinal Plants Research* 4.25 (2010): 2787-2812.

6. Nabukenya I., *et al*. "Ethnopharmacological practices by livestock farmers in Uganda: survey experiences from Mpigi and Gulu districts". *Journal of Ethnobiology and Ethnomedicine* 10 (2014): 9.

7. Farombi EO and Owoeye O. "Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcina bi flavonoid". *International Journal of Environmental Research and Public Health* 8.6 (2011): 2533-2555.

8. Bosch CH., *et al*. Vegetables of Tropical Africa. Conclusions and Recommendations Based on PROTA 2: 'Vegetables'. PROTA Foundation, Wageningen, Netherlands. 10 (2005): .

9. Ndaeyo NU. "Assessing the contributions of homestead farming to food security in a developing economy: a case study of South eastern Nigeria". *Journal of Agriculture, Forestry and the Social Sciences* 3 (2007): 11-16.

10. Igbinosa OO., *et al*. "Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas Linn". *African Journal of Pharmacy and Pharmacology* 3.2 (2009): 058-062.

11. Abdullahi MI., *et al*. "Preliminary phytochemical and antimicrobial investigations of leaf extracts of Ochna schweinfurthiana (Ochnaceae)". *African Journal of Pharmacy and Pharmacology* 4 (2010): 083-086.

12. AOAC. Official methods of analysis of the association of official analytical chemists. 5th Edition. Association of official analytical chemist's Inc 1 (1990).

13. Marcrae RR and Joslyn MA. "Antinutrient". In: Encyclopedia of Food Science, Food Technology and Nutrition. 7 (1993): 4514-4593.

14. Mbah JA., *et al*. "Bioassay-guided discovery of antibacterial agents: in vitro screening of Peperomia vulcanica, Peperomia fernandopoioana and Scleria striatinux". *Annals of Clinical Microbiology and Antimicrobials* (2012): 10.

15. Eloff JN. "Which extractant should be used for the screening and isolation of antimicrobial components from plants?" *Journal of Ethnopharmacology* 60 (1998): 1-8.

16. Allero AA and Afolayan AJ. "Antimicrobial activity of Solanum tomentosun". *African Journal of Biotechnology* 5 (2006): 269-272.

17. Kubmarawa D., *et al*. "Amino Acid Profile of Two Non-conventional Leafy Vegetable, Sesamum and Balanites aegyptiaca". *African Journal of Biotechnology* 7.19 (2008): 3502-3504.

18. Ladeji O., *et al*. "Level of antinutritional factors in vegetables commonly eaten in Nigeria". *Journal of Natural Science* 7 (2004): 71-73.

19. Nkafamiya II., *et al*. "Nutritional status of non-conventional leafy vegetables, (Ficus asperifolia and Ficus Sycomorus". *African Journal of Biotechnology* 4.3 (2010) 104-108.

20. Watson S. "What you should know about calcium oxalate crystals". (2018).

21. Oke OL. "Chemical studies on the more commonly used vegetables in Nigeria". *African Science* 11 (1969): 42-48.

22. Soetan KO., *et al*. "The importance of mineral elements for humans, domestic animals and plants: A review". *African Journal of Food Science* 4.5 (2010): 200-222.

23. Erdman JN. "Oily seed phytates nutritional implications". *JOCS* 56 (1979): 736-741.

24. Bagepallis S., *et al*. "Tannin contents of foods commonly consumed in India and Its influence on ionisable iron". *African Journal of Food Science* 33 (1993): 89-96.

25. Aletor VA. "Allelochemicals in Plant Food and Feeding stuffs I. Nutritional, Biochemical and Physio pathological aspects in Animal Production". *Veterinary and Human Toxicology* 35.1 (1993): 57-67.

26. Sotelo AE., *et al*. "Nutritional value and content of antinutritional compounds and toxics in ten wild legumes of Yucatan peninsula". *Plant Foods for Human Nutrition* 47 (1995): 115-123.

27. Umaru HA., *et al*. "Levels of antinutritional factors in some wild edible fruits of northern Nigeria". *African Journal of Biotechnology* 6.16 (2007): 1935-1938.

28. Chima CE and Igyor MA. "Micronutrients and anti-nutritional contents of selected tropical vegetable grown in south-Eastern Nigeria". *Nigerian Food Journal* 25.1 (2007): 111-117.

29. Nkafamiya II., *et al*. "Nutrient content of seeds of some wild plants". *African Journal of Biotechnology* 6.14 (2007): 1665-1669.

30. Abidemi TA., *et al*. "Nutrient content and anti-nutrition factors in shea butter (Butryospermum parkii) leaves". *African Journal of Biotechnology* 8.21 (2009): 5888-5890.

31. Amoo IA., *et al*. "Proximate Composition, Nutritional valuable Minerals, protein. functional Properties and Anti-Nutrient contents of Mucuna Preta, Mucuna Ghana and Mucuna Veuracruz Mottle". *Pakistan Journal of Nutrition* 8.8 (2009): 1204-1208.

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