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Nutritional Characteristics, Phytochemical and Antimicrobial Study of *Vaccinium parvifolium L. (Ericacea)* Leaf Protein Concentrates

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Abstract: *Problems associated with protein malnutrition are still prevalent in third-world countries, leading to a constant search for plants that could serve as nutrients and medicinal purposes. Huckleberry is one of the plants that has been proven useful locally in the treatment of numerous ailments and diseases. A fresh sample of the plant (Vaccinium pavifolium) was collected from a vegetable garden situated near the Erelu dam of the Emmanuel Alayande University of Education Campus, Oyo. The sample was authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan. The leaves of the plant were plucked and processed for leaf protein concentrates before proximate composition, mineral analysis phytochemical and antimicrobial properties were determined using a standard method of analysis.The results of proximate constituents showed; moisture content; 9.89±0.051g/100g, Ash; 3.23±0.12g/100g, crude fat; 3.96±0.11g/100g and 61.27±0.56g/100g of Nitrogen free extractive. The mineral analysis of the sample showed; Mg; 0.081±0.00mg/100g, Ca; 42.30±0.05mg/100g, Na; 27.57±0.09mg/100g, K; 6.81±0.01mg/100g, P; 8.90±0.03mg/100g, Fe; 0.51±0.00mg/100g, Zn; 0.021±0.00mg/100g, Cd; 0.04±0.04mg/100g, Pb; 0.002±0.00mg/100g, Cr; 0.041±0.00mg/100g Cadmium and Mercury were not detected in the sample. The result of phytochemical analysis of leaf protein concentrates of the Huckleberry showed the presence of Alkaloid, Saponin, Flavonoid, Tanin, Coumarin, Steroids, Terpenoids, Cardiac glycosides, Glycosides, Quinones, Anthocyanin, phytosterols, and phenols. Ethanolic extracts of the Vaccinium parvifolium L. leaf protein concentrates showed that it contains bioactive compounds that are capable of combating the following microorganisms; Staphylococcus aureus, Streptococcus pyogenes, Streptococcus faecalis, Pseudomonas aeruginosa, Klebisialae pneumonia and Proteus mirabilis. The results of the analysis of Vaccinium parvifolium L. leaf protein concentrates showed that the sample contains valuable nutrient and mineral constituents, and phytochemical compounds that could make the sample useful for medicinal activities.*

Keywords: *vaccinium parvifolium*, leaf protein concentrates, phytochemical analysis, mineral composition, antimicrobial properties, nutritional assessment

INTRODUCTION

The problem associated with food shortage is still prevalent in some countries of the world (Nigeria inclusive) leading to malnutrition and widespread deficiency diseases. This has led to a constant search for reliable sources of dietary ingredients that could serve as alternative substitutes that could partly or completely replace conventional food and feeding stuff in humans and livestock diets (Nwokoro 2015 and Nwokoro *et al.*, 2020). The plant has been the source of supply of human need from primitive time, it has been a source of food cloth, shelter and medicine. The problem of protein shortage from animals has led nutritionists to evaluate the nutritional composition of different types of plants (green vegetables, trees and medicinal plants) to bring out better alternative sources of protein from plant materials and to evaluate the nutritional, mineral and medicinal potential of the benefits that could be derived from most plants that have not been given proper attention by man, especially the leaf protein content of most medicinal plants.

Vaccinium parvifolium L. (Ericacea) is a globally widespread and genus of numerous species. The plant has been reported for its various beneficial health benefits including its antioxidant, antimicrobial, anti-inflammatory and anti-protective effects against diabetes, obesity, cancer, neurodegenerative diseases and cardiovascular disorders (Martau, *et.al.,* 2023). The plants' prevalence and commercial value have been explored leading to reports of increasing byproducts from the plant, especially in the area of its phytochemical constituent.

However, the search conducted on existing literature on this plant (*Vaccinium parvifolium*) showed no research on the leaf protein concentrates of this plant. The current study therefore aimed at evaluating the proximate composition, mineral constituents, phytochemical and antimicrobial activities of leaf protein concentrates from *Vaccinium parvifolium*. Due to the high tendency of separating green crops using mechanical methods into two fractions, Protein–rich plant juice for monogastric animals and fibrous pressed pulp for ruminants. Further processing of the green juice gives leaf protein concentrates and deproteinized juice. This has recently led to renewed interest in leaf protein concentrates to reduce the use of human-edible vegetable protein sources (Paengkoun 2010). However, the production of leaf concentrates through the process of leaf fractionation offers a considerable contribution to the alleviation of the problems of protein shortage and widespread deficiencies

MATERIALS AND METHOD

Sample Collection and Sample Processing

Fresh green leaves of the *Vaccinium parvifolium* were obtained from a vegetable garden situated very close to Erelu dam, of Emmanuel Alayande College of Education, Oyo. Oyo State. The leaves were washed with distilled water and pulped by passing it through the locally produced Mincer. The pulp was collected and strained through a cotton cloth followed by a screw press. The green juice obtained from straining the pulp through the cotton cloth was heated between 85℃ – 90℃ by steam injection, which resulted in the coagulation of all the protein present within the pulp. The Coagulum was then centrifuged from the rest of the solution, pressed, pulverized, air-dried and stored at 4℃ in the refrigerator for further chemical analysis

Proximate analysis

Proximate contents of the leaf protein concentrates of the selected plant samples were determined using the various methods described by the Association of Official Analytical Chemists (AOAC, 2005) to evaluate the approximate constituents of fats, fibre, protein and ash while the carbohydrate present in the samples was

determined by subtracting the summed up percentage composition of other proximate composition for each part of the selected sample.

Analysis of Minerals Content

Five grams (5g) of the sample was ashed in a muffle furnace at 550ºC for 12 hrs and the resulting ash was cooled in desiccators. The ash was dissolved in 2ml of concentrated HCl, and a few drops of concentrated HNO_3 were added, the resulting solution was evaporated almost to dryness in a water bath. The content was diluted to the mark level in a 100-ml volumetric flask with distilled water. The Buck scientific atomic absorption spectrophotometer. **(**Buck Scientific Model: 210 VGP and Flame Photometer FP 902) was used to determine each metal reported for the sample after the appropriate dilutions were made for each element.

Phytochemical Analysis

The method described by Lakshmi and Nair (2017) was used to determine preliminary phytochemical constituents of the five plant samples to detect the presence or absence of secondary metabolites in n-hexane. 10.0g of the selected plant samples was dissolved in 100 ml of each solvent (n-hexane, ethyl acetate, and ethanol), to give crude extracts of resulting solid brown residue obtained was kept in desiccators for qualitative phytochemical analysis.

Test for Phenols

20cm^3 of the extracts of the selected sample were treated with 5cm^3 of concentrated sulphuric acid and drops of sodium nitrate ($NaNO_3$). 2cm^3 of sodium hydroxide was added to each of the mixtures. The formation of blue precipitate indicated the presence of phenols. (Iwu *et al*., 2018).

Test for Glycosides

The Glycoside test was carried out using the method reported by Gupta *et al*., (2013). 20cm^3 of the extract of *Vaccinium parvifolium* leaf protein concentrates obtained from the process described above was heated for 5 minutes in a water bath and filtered through a Gem filter paper (12.5cm). The following tests were carried out with the filtrate.

(a) 0.2cm^3 of Fehling's solutions A and B was mixed with 5cm^3 of the filtrate until it became alkaline (tested with litmus paper). A brick-red colouration on heating showed a positive result.

(b) Instead of water, 15cm^3 of 1.0M sulphuric acid was used to repeat the above test and the quantity of precipitate obtained compared with that of (a) above. High precipitate content indicates the presence of glycoside while low content shows the absence of glycoside.

Test for Tannins

The method described by Ejikeme *et al*., (2014) as modified by Sodamade et al., 2023 was used to determine tannin content in this study. 10g of the selected plant samples was weighed into a test tube and boiled for ten (10) minutes in a water bath containing 90 cm^3 of water. Filtration was carried out after boiling using number 42 (125mm) Whatman filter paper. To 5cm^3 of the filtrate, three (3) drops of 0.1% ferric chloride was added. A brownish-green or a blue-black colouration showed a positive test.

Test for Saponins

The method used to determine qualitative test of saponin in each of the sample is as reported by Ejikeme *et al*., 2014. 100cm^3 of distilled water was added to 10g of the powdered samples and boiled for ten (10) minutes in the water bath and filtered using Whatman filter paper number 42 (125mm). A mixture of distilled water

($5cm^3$) and the filtrate ($10cm^3$) was agitated vigorously to obtain stable and persistent froth. The formation of the emulsion after three (3) drops of olive oil were added indicates a positive result (presence of saponin).

Test for Flavonoids
The method of Sofowara, (1993) and Harborne, (1973) was used for qualitative determination of flavonoids in this study as described by Sodamade et al., 2024. 10g of the sample was weighed into a beaker extracted with $60cm^3$ of distilled water for two (2) hrs and filtered with Whatman filter paper number 42 (125mm). The $5cm^3$ of 1.0M dilute ammonia solution was added to $10cm^3$ of the aqueous filtrates of the crude extract followed by the addition of $5cm^3$ of concentrated tetraoxosulphate (VI) acid. The appearance of yellow colouration which disappeared on standing shows the presence of flavonoids.

Test for Phlobatannins
The analytical method used for this determination was described by Ejikeme *et al*., (2014). 10g of the sample was weighed into a beaker, $60cm^3$ of distilled water was added. After 24 hrs of extraction, aqueous extract ($10cm^3$) of the powdered sample was boiled with $5cm^3$ of 1% aqueous hydrochloric acid. The deposit of red precipitate showed a positive test.

Test for Terpenoids
The method reported by Ejikeme *et al*., (2014) was used to determine terpenes present in the selected sample. 10g of the powdered samples was weighed into a beaker and extracted with $100cm^3$ of water which lasted for 2 hrs. A mixture of chloroform ($2cm^3$) and concentrated tetraoxosulphate (VI) acid ($3cm^3$) was added to $5cm^3$ of the extract to form a layer. The presence of a reddish-brown colouration at the interface shows positive results for the presence of terpenoids.

QUANTITATIVE ANALYSIS

Determination of Tannin content
The value of tannins present in the sample was determined by spectrophotometric method, used as described by Gupta *et al*., (2013). 5g of the selrcted plant sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and stirred for one (1) hour. The sample was filtered into a 100ml volumetric flask and made up to mark. 5ml of the filtered sample is then pipetted out into a test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008M $K_4Fe(CN)_6 3H_2O$. The absorbance of the sample was measured with a spectrophotometer at 395nm wavelength within 10min.

Determination of Saponin content
The method described by Ejikeme *et al*., (2014) and Obadoni and Ochuko (2002) was used for the determination of saponin. 10g of the powdered sample was dissolved in 100ml of 20% aqueous ethanol. The dissolved samples was stirred vigorously at about $55^{\circ}C$ for 4 hrs. The resulting mixture was filtered, and the residue was extracted with another 200ml of 20% ethanol. The combined extracts was concentrated to 40ml over a water bath at about 90^0C. The concentrate was extracted with 200ml of diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times followed by the addition of 60ml of n-butanol. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the residue was dried in an oven to a constant weight and the saponin content was calculated as a percentage.

Determination of Alkaloid

The total alkaloid contents of the sample was determined by method described by Manjunath *et al*., (2012). This method is based on formation of green colouration. 5ml of the sample extract was dissolved in 2M HCl and then filtered; 1ml of this solution was transferred to a separatory funnel and washed with 10ml chloroform. The pH of phosphate buffer solution was adjusted to neutral with 0.1M NaOH. One ml of this solution was transferred to a separating funnel and then 5ml of phosphate buffer and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in a 10ml volumetric flask and diluted to the marked volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm.

Determination of Flavonoid content

10g of the plant samples was weighed into a 100ml plastic bottle and extracted repeatedly with 100ml of 80% aqueous ethanol at room temperature. It was filtered with Whatman No 42-filter paper into a 100ml flask. This filtrate was transferred into a crucible dish and evaporated to dryness over a water bath. This was further dried in an oven at 60°C for 30 minutes and cooled in a desiccator. The crucible and the content was weighed and recorded (Ukpabi *et al*., 2014).

Determination of Total Phenolic Content

The fat-free of the selected sample was boiled with 50ml of ether for 15 minutes. The extract (5ml) was pipette into a 50ml flask and 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated aryl alcohol were also added. The sample was left to react for 30 minutes for colour development after which the absorbance of the solution was read using a spectrophotometer at 505nm wavelength. A blank solution of the extract was used for background subtraction. A standard phenol was prepared as 0.005mg/l and absorbance measured. The total phenolic content was expressed as mg/l per100g.

Determination of Oxalate by Titration Method

The method used for this determination involved three major steps: digestion, oxalate precipitation and permanganate titration (Emmanuel and Deborah 2018).

(i) Digestion:

i. 20g of sample was suspended in 190ml of distilled water in a 250ml volumetric flask.
ii. 10ml of 6M HCl was added and the suspension digested at 100^0C for 1 hour.
iii. The solution was cooled, and then made up to 250ml mark before filtration

(ii) Oxalate Precipitation

Duplicate portions of the filtrate was measured into beakers and four drops of methyl red indicator added. Then NH_4OH solution was added (drop wise) until the test solution changed from pink to faints yellow colour (pH4.0-4.5). Each portion was then heated to 90°C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was against heated to 90^0C and 10ml of 5% $CaCl_2$ solution was added while being stirred constantly. The solution was then heated and left overnight at 25°C, it was then centrifuged at 2500rpm for 5minutes. The supernatant was decanted, and the precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution.

(iii) Permanganate Titration

At this point, the total filtrate resulting from digestion of 2g of sample was made up to 300ml. aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$

solution to a faint pink colour which persisted for 30 seconds. The calcium oxalate content was calculated using the formula:

$$\frac{T \times (Vme)(Df)}{(ME \times M_F)} \times (Mg / 100g)$$

Where:

T is titre of $KMnO_4$ (ml)
V_{me} is the volume-mass equivalent
Df is the Dilution factor = Vt/A
Where Vt is the total volume of filtrate (300ml) and A is the aliquot used i.e 250ml,
ME is the molar equivalent of $KMnO_4$ in oxalate and
M_F is the mass of sample used.

Determination of Phytate

The method used to determine phytate was described by the Young and Greaves method and used as adopted by Emmanuel and Deborah (2018). 8g of the sample was weighed into 250ml conical flask, and soaked in 100ml of 20% concentrated HCl for 3 hrs, the sample was then filtered. 50ml of the filtrate was measured into a 250ml beaker and 100ml distilled water was added to the sample. 100ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g of iron per ml.

Calculation:

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

Table 1

Proximate	Value g/100g
Moisture	9.89±0.051
Ash	3.23±0.12
Fat	3.96±0.11
Protein	15.71±0.30
Crude fibre	5.93±0.11
Carbohydrate	61.27±0.56

Table 2: Minerals

Mineral	Mg	Na	K	Zn	P	Fe	Cr	Ni	Cd	Pb	Ca
Conc. Mg/100g	0.08±0 .00	27.57± 0.09	6.81± 0.01	0.02± 0.00	8.90± 0.03	0.51± 0.00	0.04± 0.00	0.00± 0.00	0.04± 0.47	0.002±0 .00	34.28± 0.10

Table 3: Phytochemical Screening Qualitative

PARAMETERS	INDICATION
Saponin	+
Alkaloid	+
Flavonoid	+
Tannin	+
Coumarin	+
Steroid	+

Terpenoid	+
Cardiac Glycosides	+
Glycosides	+
Quinones	+
Anthocyanin	+
Phytosteroids	+
Phenols	+

+= presence of parameters measured.

Table 4: Qualitative Analysis

Parameter	Value
Saponin	1.37±0.04
Alkaloid	4.77±0.13
Flavonoid	48.19±0.31
Phenol	18.11±0.15
Tannin	6.09±0.16
Phytate	1.03±0.63
Oxalate	0.98±0.03

Table 5:

Microbes	Antimicrobial activity of the extract
S.A	+
NG.	+
ST.P	+
STP.	+
PSA	+
KP	+
Pr.M	+
C.K	+
C.A	+
CV	+

The results of the proximate composition of huckleberry leaf protein concentrates was presented in Table 1 above. The sample contains 9.39±0.05g/100g of moisture content. The value is low compared to the 26.70g/100g reported for *C. bundue* by Ojelere, 2016. The moisture content of this sample is also low compared with 18.63±2.11g/100g reported for the leaf of *Costus afer* (*Ginger lily*) by Anyasor et.al, 2014. The low value of moisture in the sample implied the long shelf life of food, the moisture content of samples influences the stability of the sample at room temperature. It also implies the easy absorption and assimilation rate of the samples when used as food ingredients for man or animals. The amount of moisture suggested for most green vegetables ranged between 6% and 15% indicating that the moisture content of *Vaccinium parvifolium* leaf concentrates contain nutritionally favoured moisture qualities.

The ash content of *Vaccinium parvifolium* leaf protein concentrates is 3.23±0.12g/100g. The values of ash in any food substance determine the level of availability of inorganic substances (Hofman et.al, 2013). The value of ash in this sample is high compared to 0.67±0.06g/100g, 0.64±0.07g/100g, 0.63±0.04g/100g and 0.50±0.06g/100g reported for Mango, Banana, Guava and Papaya respectively by Ferdaus et.al, 2020. The value of ash in this sample implies the presence of mineral elements in moderate amounts in the leaf protein concentrates of *Vaccinium parvifolium.*

The crude fat content of *Vaccinium parvifolium* leaf protein concentrates is 3.96±0.11g/100g. The value of crude fat in this sample is slightly higher than 3.17±0.47g/100g reported for *Maerua grassifolia* leaves but low compared with 7.30±0.26g/100g reported for *Brysocarpus coccineus* by Sulaiman et.al, 2019. The value of crude fat in this sample agreed with a general observation that most plants and green vegetables are low-fat-containing samples that would be of good nutritional choice for those who want to cut down on high dietary fat (Awol, 2014). Fat plays a significant role in the level of blood pressure in the cellular part of the body (Analifo, 2006). It also enhances the flavor, palatability and mouth feel of food (Kassegn, 2018).

The crude protein value of *Vaccinium parvifolium* leaf protein concentrates is 15.71±0.30g/100g. The values of crude protein present in this sample are low compared with 80.05±0.85g/100g, 30.95±0.07g/100g and 32.22±0.02g/100g reported for egg white, *Amarathus hybridus and Amaranthus cruentus* vegetable respectively by Kamelea *et al.,* 2016. To enhance the leave of protein present in this sample, the sample could be mixed with protein-rich food protein is required for the formation of antibodies that help the body to fight infection. It is an essential ingredient of amino acids which assist in the formation of hormones and enzymes (Ahmed, 2014).

Crude fibre content of *Vaccinium parvifolium* is 5.93±0.11g/100g. The crude fibre of this sample is greater than 2.55±0.70g/100g, 2.81±0.26g/100g and 3.01±0.88g/100g reported for the leaf, root and stem of *Maesobotrya barteri* respectively by Etukudo and Osim (2018). Crude fibre assists in the prevention of heart diseases, colon diseases, colon cancer and diabetes. It also assists in keeping the digestive system healthy and functioning properly (Kassegn 2018 and Ahmed 2014).

The Nitrogen free extractive present in the sample is 61.27±0.56g/100g. The value is the highest of the other parameters of proximate constituents of this plant sample. The value obtain for NFE is higher compared with 53.10±0.46 reported for the leaf of *Tridax procumbens* by Runde et.al, 2020. It is also greater than values reported for *Dracaena manni* and *Dracaena arborea* leaf stem and bark by Ilodibia et.al, 2014.

The results of the mineral composition of *Vaccinium parvifolium* leaf protein concentrates were showed in Table 2. The sample contains 34.28±0.10mg/100g. The concentration of calcium in this sample is lower than 68mg/100g and 124mg/100g reported for pigeon pea and Ima bean respectively by Arinatan et.al., 2003. Calcium has been reported to play a strong role in building strong bones and good teeth formation (Gbadamosi and Oloyede 2014). Calcium also assists in the assimilation of vitamin D in the body and is needed for effective communication between the nerves and the brain (Emeka, et.al, 2014). The recommended daily allowance of calcium is 10mg per day for infants and 800mg per day for adults male and female.

The magnesium concentration of *Vaccinium parvifolium* leaf protein concentrates is 0.08±0.00mg/100g. The amount obtained is low compared with 2.93±0.02mg/100g reported for *Allium sativa* by Kizil *et.al.,* 2010 respectively. Magnesium is required in the body for regulating Osmotic pressure and the amount of blood plasma present in the extracellular fluids at optimal value. It is also an essential material in the formation of

bones, cartilage and teeth in human beings and mammals. It also assists in the regulation of normal enzymatic activities in the body (Hassan *et al.,* 2015). A deficiency of magnesium in human being always results in diarrhea, vomiting, neuromuscular hyperirritability and cardiac arrhythmia. A high amount of this sample will have to be utilized to meet the optimum required dosage of magnesium.

The sodium content of *Vaccinium parvifolium* is 27.57±0.09mg/100g. The value is high compared with 5.47±0.51mg/100g, 6.51±0.36mg/100g and 4.26±0.45mg/100g reported for the leaf, stem and root of *Maesobotrya barteri* respectively by Etukudo and Osim 2018. Sodium is one of the dietary minerals that assist in the regulation of plasma volume and acid-base balance in the human body. It is the principal cation of extracellular fluids and it is required for the absorptive processes of monosaccharides amino acids and bile salts. Too much sodium tends to cause renal disorders, an increase in blood pressure levels and cardiovascular disorders (Haruna *et al.,* 2015). The level of sodium concentration in this sample is within the tolerable amount since it can contribute 5.5% to the recommended daily allowance.

The potassium concentration of *Vaccinium parvifolium* leaf protein concentrates is 6.81±0.01mg/100g, the value is low compared to 438.00±1.29mg/100g, 114.00±1.17mg/100g and 96.00±0.16mg/100g reported for the leaf, stem and root of *Miletia aboensis* by Oku *et al.,* 2017. The recommended daily allowance of potassium for adults is 200mg per day which implies that *Vaccinium parvifolium* leaf protein concentrates can contribute 3.4% of the required dietary allowance. Potassium is reported to assist sodium in proper fluid balance in the human body, it aids nerve transmission and muscle contraction (Haruna *et al.,* 2015).

The concentration of dietary zinc in the leaf protein concentrates of *Vaccinium parvifolium* is 0.02±0.00mg/100g. The value of zinc in the sample is low compared with 11.34±1.16mg/100g reported for *Fiscus benghalensis* seed by Govindan and Shoba (2015). The recommended daily allowance of zinc is 8mg per day for adult women and 11mg per day for adult men (Fagbohun *et al.,* 2012). This implies that a high amount of the selected sample would be required to meet the required daily requirement. Zinc has been reported to aid proper hormonal balance, it is an essential requirement that maintains various reactions in the body. It is required for the growth of tendons, ligaments and body tissues (Saji *et al.,* 2014).

The value of phosphorus concentration present in the leaf concentrates of *Vaccinium parvifolium* is 8.90±0.03mg/100g. The concentration of phosphorus present in this sample is low compared to 24.91mg/100g reported for *Urena lobata* leaves by Njoku *et al.,* 2020. Phosphorus is one of the mineral elements that assist magnesium in healthy bone and teeth formation. It gives necessary reinforcement for cells in the body and maintains the acid-base balance of cell cytoplasm in human beings (Afolabi et.al, 2012).

The concentration of iron in the leaf protein concentrates is 0.51±0.00mg/100g. The value is low compared to 84.4mg/100g reported for *Helminth ostachys* spp by Awol 2014. The recommended daily allowance of iron is 300mg for males and 20mg per day for females at age 20 and children between ages 10 and 17. Iron is required for the formation of heamoglobin, myoglobin and some enzymes in man.

The chromium concentration of *Vaccinium parvifolium* leaf protein concentrates is 0.04±0.00mg/100g. the value is lower than 17.11±1.14mg/100g reported for *Fiscus benghalensis* seed by Govindon and Shoba 2015. Chromium helps insulin to regulate blood sugar levels in human beings, it also functions as a powerful antioxidant that assists in the maintenance of RNA molecules. The concentration of Nikel in the sample is below the defecting limit while cadmium and lead concentrations are 0.04±0.047 and 0.002±0.00 respectively.

The results of Qualitative and Quantitative phytochemical analysis are presented in Table 3 and Table 4. The results of the qualitative phytochemical analysis showed the presence of saponin, Alkaloids, Flavonoids, Tannin, Coumarin, Steroids, Terpenoids, cardiac glycosides, glycosides quinines, anthocyanin, phytosterols and phenols. The results showed that the sample would be beneficial in the treatment of some diseases due to the presence of phytochemical compounds of high medicinal value.

The results of quantitative phytochemical analysis on the selected samples were shown in Table 4. The saponin detected in *Vaccinium parvifolium* leaf protein concentrates is 1.37±0.04mg/100g. The Saponin content of this sample is lower than 2.27±0.03mg/100g reported for *Jathropha curcas* by Asuk et.al, 2015. The value is however higher than 0.54±0.01mg/100g reported for *Carpolobia lutea* by Olayinka et.al, 2019. Saponin assists in knocking out tumor cells through its diverse biological activity and helps the body fight against microbial infections (Okwu and Ndu 2006).

The Alkaloid concentration present in *Vaccinium parvifolium* leaf protein concentrates is 4.77±0.13mg/100g, the values obtained for this sample are higher than 2.52±0.21mg/100g reported for *Maesobotrya barteri* by Etukudo and Osim 2018. Alkaloids have been reported as an important class of phytochemical compounds that have numerous phytochemical applications. Alkaloids are known to exercise antioxidative activities, they serve as protection against allergies, inflammation, platelet aggregations, hepatic toxicity and microbes (Sibi et.al, 2014).

Flavonoids concentration present in leaf concentrates of *Vaccinium parvifoliumis 48.19*±0.31mg/100g. The values are higher than 1.34±0.02mg/100g and 1.21±0.02mg/100g reported for the stem and leaf of *Urena lobata* respectively by Abi and Omuha 2014. Flavonoids have antioxidant properties that assist the human body in the prevention of various types of cancer (Ben et.al, 2013). It also protects the human body against platelet aggregation, Ulcers, virus and boosts the production of detoxifying enzymes in the body (Okwu and Ndu 2006). Flavonoids have the highest value among all the phytochemical parameters analyzed in the selected samples.

The phenol concentration of the leaf protein concentrates of *Vaccinium parvifolium* is 18.11±0.13mg/100g. The value of phenol in this sample is higher than 0.04±0.01mg/100g and 0.05±0.01mg/100g reported for the leaf and root of *Capolobia lutea* respectively by Olayinka et.al, 2019. The value of phenol in this sample is also high compared to 2.77±0.55mg/100g reported for the stem of *Jathropa curcas* by Asuk et.al, 2015.

Phenols have been reported to provide some health benefits like radical scavenging activity, anti allergic and anti-inflammatory properties (Sharma et.al, 2019). This may be the reason why the leaf of *Vaccinium parvifolium* is used as an anti-inflammatory substance due to the high content of flavonoids and phenols it contains. The concentration of Tannin present in the leaf concentrates of *Vaccinium parvifolium* is 6.09±0.16mg/100g. The value is low compared to 8.94±0.06mg/100g but higher than 4.78±0.31mg/100g reported for the stem of *Urena lobata* and the leaf of *Carpolobia lutea* by Njoku et.al, 2020 and Agbafor et.al, 2015 respectively. Tannin has been reported to be useful in the treatment of hemorrhage, diarrhea, diabetes, and fast healing of wounds and bruises (Kumara and Jain 2015).

The phytate and oxalate composition of *Vaccinium parvifolium* leaf concentrates are 1.103±0.63mg/100g and 0.98±0.03mg/100g respectively. oxalate is one of the anti-nutrient content in plant samples that showed a high tendency to bind metals especially calcium, iron and magnesium resulting information of insoluble salts making them unavailable for body use (Abdoulaye *et al.*, 2011). Phytate on the other hand has been confirmed

to hinder the absorption and digestion of some mineral elements it lead to low absorption and digestion of protein and lipid in the body (Kumar *et al.,* 2010). The amount of oxalate and phytate in this sample is low since it does not exceed the recommended dose of 2.5g/100g and 5g/100g which may impose health hazards in food (Morale *et al.,* 2014).

The result of the antimicrobial activity of *Vaccinium parvifolium* leaf concentrates is shown in Table 5. The extracts from the leaf concentrates of the selected sample showed good activity against the selected pathogens, indicating the broad spectrum activity. The zone of inhibition from the extracts against test isolates (28mm) was lesser than the one of inhibition from doxycycline (38mm) used as a positive control in this study.

In conclusion, the result of the analysis of leaf protein concentrates of *Vaccinium parvifolium* showed that the sample is a veritable source of nutrient and mineral constituents. The sample also contains phytochemical compounds that possess useful bioactive characteristics of high medicinal value. The results of antimicrobial activities from the extracts from the sample also indicate that the bioactivity of the plant is high towards the eradication of some disease-causing microorganisms.

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