

Assessment of Proximate, Mineral, Phytochemical and Antimicrobial Properties of *Brassica oleraceae*L. Leaf Concentrates

ABSTRACT

Food insecurity, ill health due to microbial infection and malnutrition have led to the search for alternative and affordable measures to address food shortage problems and associated health issues worldwide. This provides an opportunity to investigate the use of plants as a remedy. A fresh sample of *Brassica oleracea* was selected for the study; the sample was obtained and identified at the University of Ibadan, washed before being processed into the leaf concentrates and assessed for proximate, mineral, phytochemical and antimicrobial potentials using the standard methods. The results of proximate analysis in g/100g showed; moisture; 8.80 ± 0.16 , Ash; 1.38 ± 0.10 , crude fat; 4.66 ± 0.12 , crude protein; 35.52 ± 0.3 , crude fibre; 3.16 ± 0.06 and NFE; 46.45 ± 0.23 . The mineral analysis in mg/100g showed Na; 35.81 ± 0.23 , K; 3.37 ± 0.01 , Ca; 24.71 ± 0.02 , Mg; 0.24 ± 0.00 , P; 0.91 ± 0.00 , Fe; 0.90 ± 0.01 , Zn; 0.50 ± 0.00 , Cr; 0.10 ± 0.00 , Ni; 0.00, and Cu; 0.02 ± 0.01 . The sample contains some bioactive phytochemical compounds like steroids, anthraquinone terpenes, and coumarin. The sample extracts also showed antimicrobial potential against the ten selected microbes. The study conducted so far revealed that the sample will be viable ingredients in food formulation, pharmaceutical raw materials and alternative sources of protein.

KEYWORDS: Leaf protein, Antimicrobial Potential, Phytochemical analysis, Proximate composition

INTRODUCTION

Plants and plant products have been used for many decades as **remedies for combating problems associated with malnutrition and widespread deficiency diseases**. They have served as the source of food, shelter and medicine according to the type of plant species involved. Medicinal plants are of great importance to the health of individuals and communities because a large populace of people in developing countries depends on plant-based drugs to combat ailments and diseases. This is due to their easy availability, therapeutic potential, least side effects and minimal cost (Velmuruganand Anand, 2017). However, **the gradual decline in protein supply due to high-cost animal-sourced proteins leading to calorie malnutrition and widespread deficiency diseases coupled with the global increase in inflation has drifted the focus of nutrients** toward protein from plant sources. Continued interest in plant analysis has gradually been stimulated in recent years as a result of a progressive increase in problems associated with global food shortage, overpopulation and economic depression. Also, many researchers have recently explored the nutritional capability of leaf concentrates of some green vegetables (Deshmukh and Waghmode 2011) cassava leaves (Gundersen *et al.*, 2022) and bioactive riched plant leaves.

Nowadays most leaves from plants are mostly treated as waste materials except green vegetables. Yet this readily available biomass are rich source of protein with a balanced amino acid content, antioxidants, minerals as well as bioactive compounds of high medicinal activities with low content of anti-nutritional substances compounds factors. Therefore the objective of this present study is to evaluate the nutrient composition phytochemical constituents, mineral composition and antioxidant potential of *Brassicaoleracea* Leafconcentrates.

BrassicaoleraceaL is a plant species from the family of *Brassicaceae* that consists of many other cultivars used as vegetables such as cabbage, broccoli, cauliflower, kale, *Brussels sprouls*, collard greens and so on. It is a plant grown for its edible leaves. It is usually **grown** on rocky sea cliffs along the coastline. The leaf is eaten as a vegetable throughout the world, after the removal of the petioles and the thick midribs. The leaves of the plant are often consumed because they are believed to have anticarcinogenic, antirheumatic as well as gout treatment capability (Ferdaset *al.*, 2020). The seeds of the plants are considered to be laxative, diuretic stomachic and antihelminthic. Therefore the various claims in the existing literature on the usefulness of *BrassicaoleraceaL*. leaf as a nutraceutical plant could be investigated in the leaf concentrates which is the purpose of the present investigation.



Picture 1: *Brassica oleracea*(L)

Materials and Method

Sample collection and treatment

Information on the medicinal properties and usefulness of *Brassica oleracea* L was gathered from a farmer who specialized in the commercial cultivation of the plant. The plant sample was collected from the botanical garden of the University of Ibadan. The sample was then authenticated at the Forest Research Institute of Nigeria (FRIN) after collection. The leaves of the plants were separated from the stem and washed with distilled water before processing into the leaf concentrates.

Sample Preparation

The leaves of *Brassica oleracea* were fractionated after rinsing with distilled water, followed by pulping using a laboratory mincer to rupture the plant cell. The juice was then removed hydraulically and heated to 85°C with the steam injection to coagulate the leaf concentrates. The resulting coagulum was separated by cloth filtration followed by vacuum drying at 50°C and subsequently stored at -4°C in a freezer for further analysis.

Proximate Analysis

Proximate contents of the selected plant samples were determined using the various modified methods of the Association of Official Analytical Chemists (AOAC, 2016) as described by Beshaw *et al.*, 2022 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.

Determination of moisture content

The AOAC (2016) formal analysis method was used to determine moisture content. 2 g of the leaf concentrates of the selected sample was weighed into three clean, dried, and pre-weighed porcelain crucibles. The leaf protein concentrate of *Brassica oleracea* was dried in the moisture extraction oven at 100 °C for 3 hours. The dried samples were removed from the oven, cooled in a desiccator and reweighed. This procedure was repeated until a constant weight of the samples was obtained. This analysis was carried out as weighed in triplicate and the average value obtained was recorded as moisture content. The percentage moisture content was calculated using the equation:

$$\text{Moisture (g/100g)} = \frac{W_2 - W_3}{W_2 - W_1}$$

Where W_1 = Weight of empty moisture can

W_2 = Weight of empty moisture can and sample before drying weight

W_3 = Weight of the moisture can and the sample dried to a constant weight

Determination of fiber content

Three weighings were performed on 2 grams of leaf concentrates of *Brassica oleracea* in triplicate. 200 mL of H_2SO_4 was added to the sample, heated for 30 minutes, and then filtered. After washing the residue three times with hot water, 100 mL of 2% NaOH was added to the residue, heated for 30 minutes, and then filtered. Then carefully wash the filtrate three times with hot water until the acid is free. The samples were vacuum-dried, placed in an oven at 105 °C overnight and reweighed. The residue was processed in a muffle furnace at 550 °C for 3 hours until light gray ash was formed, then weighed each time and the total fiber percentage was calculated thus:

$$\text{Crude fibre (g/100g)} = \frac{W_2 - W_1}{\text{Weight of the sample}} \times 100$$

W_2 = Weight of the crucible + sample after washing, boiling and drying

$W_3 = \text{Weight of crucible} + \text{ash of the sample}$

Determination of crude protein

The Kjeldahl method was used to determine the crude protein constituents of the leaf concentrates of *Brassicaoleracea*. 5 g of the sample in triplicate was weighed using analytical balance into the Kjeldahl digestion flask. 2 g of Kjeldahl catalyst and 200 mL H_2SO_4 were added and boiled until the content of the flask became clear and allowed to cool down. 60 mL of water and 6 drops of the mixed indicator were added and heated until all N_2 was distilled. Finally, the solution was titrated with NaOH and the crude protein content was calculated using the equation:

$$N_2(\text{g}/100\text{g}) = \frac{100 \times M \times 14 \times V_t}{W \times 100 \times V_a} \times T \times B$$

Where $W = W_t$ of sample, $M = \text{Molarity of titrant (0.02M)}$

$V_t = \text{Total digest volume (100ml)}$ $V_a = \text{Volume of digest analysed}$

$T = \text{Sample titre value}$ $B = \text{Blank titre value}$

Determination of Fat content

4 g of the leaf concentrate of *Brassicaoleracea* were weighed in triplicate into extraction thimble (w_1) and transferred into a Soxhlet extractor connected with an extraction flask filled with 250 mL petroleum ether and heated at 85 °C until the solvent was completely siphoned over, the extraction lasted 6 hours. The extraction thimble containing the sample was cooled in desiccators and reweighed. The fat content was calculated as follows:

$$\text{Fat Content (g}/100\text{g)} = \frac{W_2 - W_1}{\text{Weight of the sample}} \times 100$$

Where $W_1 = \text{Weight (g) of the empty extraction flask}$

$W_2 = \text{Weight of flask} + \text{oil extract}$

Determination of Carbohydrate

The percentage of carbohydrates present in the leaf protein concentrates of *Brassicaoleracea* leaf concentrates was calculated by using the following formula:

$$\text{Carbohydrate (g}/100\text{g)} = 100 - (\text{Crude protein (g}/100\text{g)} + \text{Crude fat (g}/100\text{g)} + \text{Crude fibre (g}/100\text{g)} + \text{Ash (g}/100\text{g)} + \text{Moisture (g}/100\text{g)})$$

Determination of Energy Value of Sample

The gross energy of the leaf protein concentrate of *Brassica oleracea* was calculated using the formula:
The gross energy value = (Crude protein (g/100g) × 4) + (Crude fat (g/100g) × 9) + (Carbohydrate (g/100g) × 4)

Determination of Mineral Composition

The dried leaf concentrate of the samples was homogenized with an electric mixer and digested in a microwave oven. Microwave digestion with mixtures of trioxonitratev acid and perchloric acid was used to solubilize leaf protein concentrate samples. This closed-vessel pressurized boiling technique has gained popularity as a simple and rapid dissolution technique that minimizes acid consumption, the risk of sample contamination and the loss of volatiles during boiling before atomic absorption spectrophotometric analysis containing 9 mL HNO₃ and 4.5 mL HClO₄. (2:1 v/v) and digested. The solution was allowed to cool at 120°C for 3 hours. Lanthanum oxide was added as a catalyst and distilled water was added during cooling to dissolve the formed precipitate. The solution was then transferred to a 50 mL volumetric flask, diluted with deionized water, and filtered through Whatman No. 42 filter paper, and the digested samples were stored in a refrigerator until the concentration of all metals in the sample solutions was determined by flame. atomic absorption Spectrophotometer equipped with deuterium arc background correctors and air-acetylene flame hollow cathode lamps.

Antimicrobial Screening of *Brassica oleracea* leaf protein concentrates Extracts

Bacterial cultures were grown in Heart Brain Expansion broth at 37°. After growing for 6 hours, each microbe was inoculated onto the surface of Mueller-Hinton agar plates at a concentration of 10⁶ cells/ml. A filter paper disc (6mm diameter) filled with extracts and ampicillin (50 uL) was placed on the surface of each inoculated plate. To evaluate the effectiveness of the method, each extract was placed in a well (50uL) made in a new plate. The plate was incubated at 37°C for 24 hours. Later, it was possible to see the blocking zones. In general, cultured bacteria with a diameter greater than 7 mm were considered sensitive to the extracts or chemicals tested.

The extracts were neutralized in a culture medium using DMSO and Tween80(Polysorbate 80). The control was the solvent used for extracts, which showed no inhibitory effect in preliminary studies. Extracts that showed antibacterial activity were later tested to determine the minimum

inhibitory concentration (MIC) for each bacterial sample. The microorganisms tested against the crude extracts from the leaf concentrates of *Brassica Oleracea* are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteins mirabites*, *Candida krusei*, *Candida albicans* and *Candida valida*. which were grown in a nutrient medium for 6 hours. Then, 100 μ L of 10⁶ cells/mL was injected into tubes containing nutrient solution supplemented with various concentrations (8 – 70 μ L) of extracts and Standard Drug (Ampicilin). After 24 hours at 37^oC, the MIC of each sample was determined by measuring the optical density 620 nm in a spectrophotometer and comparing the sample readings to the non-inoculated nutrient medium.

Table 1: Results of Proximate constituents of *Brassica oleracea* leaf concentrates

Parameter	Value g/100g
Moisture content	8.80 \pm 0.16
Ash content	1.38 \pm 0.10
Crude fat	4.66 \pm 0.12
Crude protein	35.52 \pm 0.31
Crude fibre	3.16 \pm 0.06
NFE	46.45 \pm 0.23

The results of proximate constituents of *Brassica oleracea* leaf concentrates are shown in Table 1. The moisture content of the sample is 8.80 \pm 0.16g/100g. the percentage moisture composition of this sample is in the same range as the values reported for the leaf and root of *Rumex crispus* (L) by Idris et.al 2019. The moisture value obtained for this sample does not exceed 10.g/100g reported to be a non-acceptable moisture value limit for long-term storage of flour (Adomet *al.*, 2015). The value of moisture composition in the leaf protein concentrate of this sample implies that its leaf concentrates can be milled into powder and could be stored for a long time without spoilage. The moisture value obtained for this sample also implied quick digestion and assimilation with the body.

Leaf concentrates of *Brassica oleracea* contain 1.38 \pm 0.10g/100g of Ash. The ash content of this sample is slightly high compared with 0.54 \pm 0.08g/100g and 0.45 \pm 0.04g/100g reported for bottle and pointed gourd respectively by Ferdauset *al.*, 2020. The low value of ash obtained for this sample implied the presence of minerals in a moderate amount. The value of ash in this sample of however low compared with 12.17 \pm 0.47g/100g reported for *Maer vacrassifolia* leaves by Aletan and Kwazo 2019.

The crude fat content of *Brassicaoleracea* leaf concentrates is $4.66\pm 0.12\text{g}/100\text{g}$. The crude fat of this sample is higher than $2.28\pm 0.09\text{g}/100\text{g}$ reported for seed oil of *Bouhiniatomentosa* by Ajani *et al.*, 2016. The value is however low compared with $28.2\pm 0.00\text{g}/100\text{g}$ reported for soybean seed by Ogbemudia *et al.*, 2018. Fat in food serves as an important source of energy during starvation or fasting, it also acts as an important biological and structural function of cells (Ayanda *et al.*, 2018).

The value of crude protein present in the leaf concentrates of *Brassicaoleracea* is $35.52\pm 0.31\text{g}/100\text{g}$. The crude protein values of this sample are higher than $3.35\pm 0.22\text{g}/100\text{g}$ reported for *Bryocarpuscooccineus* and $10.36\pm 0.07\text{g}/100\text{g}$ reported for *Peperomiapellucid* by (Suleiman *et al.*, 2019) Ooiet *al.*, (2012) respectively. Protein is required for the formation of hormones required for growth, repair and maintenance of body tissue. Protein is also required for the formation of antibodies that assist the human body to fight infection (Awol, 2014).

The crude fibre content of *Brassicaoleracea* leaf concentrates is $3.16\pm 0.06\text{g}/100\text{g}$. The value of crude fibre in this sample is low compared with $6.5\text{g}/100\text{g}$ and $8.5\text{g}/100\text{g}$ reported for white and purple wheat by Kassegn (2018) and $28.00\pm 0.69\text{g}/100\text{g}$ reported for *Bauhinatomentosa* seed oil by Ajani *et.al.*, (2018). Crude fibre assists the human body in the prevention of heart disease, colon cancer and diabetes, fibre in food assist in keeping the human digestive system in good condition and proper functioning (Kassegn 2018 and Ahmed 2014). A high amount of *Brassicaoleracea* leaf concentrate will be consumed to meet to required dietary fibre.

The leaf concentrates of *Brassicaoleracea* contain $46.45\pm 0.23\text{g}/100\text{g}$ of nitrogen-free extractive. The value of crude carbohydrate in this sample is higher than $42.50\text{g}/100\text{g}$ reported for *Cactuscladode* by Bakari *et al.*, (2017) but lower than $74.22\text{g}/100\text{g}$ reported for wheat by Ahmed *et al.*, 2012. Carbohydrate in food is the major source of energy, they also help to regulate nerve tissue at high concentrations Kassegn (2018). Carbohydrates assist the human body in numerous biochemical reactions that are not directly concerned with energy metabolism (Ngaha 2016). The result of carbohydrates in this sample is a reliable source of food carbohydrate.

Table 2: Mineral Composition

Mineral	Na	K	Ca	Mg	P	Fe	Zn	Cr	Ni	Cd	Pb	Se	Cu
Value	35.81	$3.37\pm$	$24.71\pm$	$0.24\pm$	$0.91\pm$	$0.90\pm$	$0.50\pm$	$0.10\pm$	0.00	0.00	$0.03\pm$	$0.00\pm$	0.02
mg/100g	± 0.23	0.01	0.02	0.00	0.00	0.01	0.00	0.00	± 0.0	± 0.0	0.00	0.00	± 0.01

The concentration of different minerals present in the leaf concentrates of *Brassicaoleracea* is presented in Table 2. The sample had $35.81\pm 0.23\text{mg}/100\text{g}$ of sodium, the values are low compared

with 84.11mg/100g and 50.55mg/100g reported for turmeric and scent leaves respectively by Paul *et al.*, 2018. The value is however higher than 13.0mg/100g and 12.00mg/100g reported for ginger leaves and bitter leaves respectively by the same authors (Paul *et al.*, 2018).

Sodium is required in the human body for acid-base balance regulation, it assists in the transport of metabolites, the transmission of nerve impulses and blood pressure regulation Murugeset *al.*, 2021. If this sample will be utilized to perform the dietary roles of sodium, an adequate quantity of the sample will be utilized. The leaf concentrates of *Brassicaoleracea* have 3.37±0.01mg/100g of dietary potassium. The value is low when compared with 7.29±0.14mg/100g, 6.74±0.20mg/100g and 5.66±0.90mg/100g reported for the leaf, stem and root of *Maesobotryabariteri* respectively by Etukudo and Osim 2018. Potassium assists the human body in preventing stroke and hypertension; it assists iron in the body in regulating herpes. It also reduces the chance of kidney stones (Anwani *et al.*, 2020). The recommended daily allowance of dietary potassium is 200mg per day for an adult male. However large quantity of *Brassicaoleracea* leaf concentrates will be required to meet the recommended daily allowance.

The concentration of calcium in the leaf concentrates of *Brassicaoleracea* is 24.71±0.02mg/100g. Calcium is required for the synthesis of vitamin D and the activation of some enzymes in the human body. Calcium is helpful in the human body for nerves and muscle function; it assiststhe human body in forming thrombin from prothrombin, (Koubova *et al.*, 2014). The value of calcium in the *Brassicaoleracea* leaf concentrates is higher than 6.77±0.21mg/100g and 8.43±0.36mg/100g reported for the leaf and root of *Maesobotryabariteri* by Etukudo and Osim (2018). The recommended daily requirement of calcium is 165 – 265mg per day (NRC, 1989). The amount of calcium present in this sample is low, required for utilization of a large amount for the recommended value to be met.

The leaf concentrates of *Brassicaoleracea* contain 10.24±0.00mg/100g of magnesium. The concentration of magnesium in this sample is higher than 4.26±0.45mg/100g and 4.69±0.54mg/100g reported for the root and leaf of *Maesobotryabariteri* respectively by Etukudo and Osim (2018). Magnesium assists in the prevention of circulatory and heart-related diseases. It assists in calcium metabolism and the prevention of cardiovascular diseases (Prasad *et al.*, 2010). The value of magnesium in this sample will be helpful in the prevention of cardiovascular disease and calcium utilization. A deficiency of magnesium in human beings always results in diarrhea, serious vomiting, cardiacarrhythmia and neuromuscular irritability (Soetanet *al.*, 2010).

The concentration of phosphorus present in the leaf concentrates of *Brassicaoleraceais* $0.91\pm 0.00\text{mg}/100\text{g}$. The value of phosphorus in this sample is higher than $0.04\pm 0.01\text{mg}/100\text{g}$ and $0.03\pm 0.01\text{mg}/100\text{g}$ that were reported by Etukudo and Osim, 2018) for the root and stem of *Maesobotryabarteri* respectively. The value of phosphorus in this sample is lower than $4.47\pm 0.23\text{mg}/100\text{g}$ and $1.33\pm 0.12\text{mg}/100\text{g}$ reported for the leaf and root of *Jatrophacurcas* respectively by Asuket *al.*, 2015.

Phosphorus assists in the synthesis of calcium. It is also responsible for the formation of good bones and strong teeth in man. Phosphorus also plays a significant role in blood clotting and muscular contraction (Anyasoret *al.*, 2014). The recommended daily allowance of phosphorus is 700mg per day for the adult. The value of phosphorus present in the leaf concentrates of *Brassicaoleraceac* cannot meet the recommended daily allowance. A huge amount of the leaf concentrates of the selected sample will be required to meet the recommended daily allowance.

Iron is required for the formation of hemoglobin in the human body, it is also essential in the formation of ligaments and tendons (Chuck and Ugochi, 2012). A deficiency of iron results in anemia, general weakness, and slow social and cognitive development. The concentration of iron in the leaf concentrates of *Brassicaoleraceais* $0.90\pm 0.01\text{mg}/100\text{g}$. The value is too low compared to the $9.35\text{mg}/100\text{g}$ reported for *Urenalobata* leaves by Njoku *et al.*, 2020. The iron content of this sample will contribute a small amount of the recommended daily allowance of 300mg for males and females of age 20 and 20mg per day required per day for the children (NRC, 1989).

The value of zinc present in the leaf concentrates of *Brassicaoleraceais* $0.50\pm 0.00\text{mg}/100\text{g}$ while the value of chromium is $0.10\pm 0.01\text{mg}/100\text{g}$. The value of the zinc in this sample is in the range of $0.43\pm 0.10\text{mg}/100\text{g}$ reported for the root of *Maesobotryabarteri* by Etukudo and Osim 2018 but lower than 2.6mg reported by Shumaiki and Mahpara (2009). The value of chromium in this sample is slightly higher than $0.010\pm 0.00\text{mg}/100\text{g}$ and $0.02\pm 0.00\text{mg}/100\text{g}$ reported for the leaf and root of *Jathropacurcas* by Asuket *al.*, 2015.

Chromium is a micromineral required for the proper function of insulin to stabilize blood sugar levels and increase muscle mass by reducing fat in the human body (Schaus 2015). Zinc on the other hand assists in the metabolism of macronutrients through some of its associated enzymes. Zinc is also known to assist DNA and RNA in cell replication and bioavailability of vitamins A and E. Nickel, cadmium and selenium were not detected in the leaf concentrates of *Brassicaoleraceaw* while lead and

copper were present in small concentrations of $0.03 \pm 0.00 \text{ mg/100g}$ and $0.02 \pm 0.01 \text{ mg/100g}$ respectively.

The results of the qualitative and quantitative phytochemical analysis of *Brassica oleracea* are contained in Table 3 and Table 4 respectively. The results of quantitative showed the presence of some vital bioactive compounds; Saponin Alkaloids, Flavonoids, Tannin, Coumarin, Steroids, Terpenoids, Cardiac glycosides, Quinones, phytosteroids and phenols while anthocyanin was not detected in the sample.

In Table 4, the value of saponin present in the selected sample is $5.76 \pm 0.07 \text{ mg/100g}$. The value of saponin in this sample is higher than $1.10 \pm 0.05 \text{ mg/100g}$, and $2.10 \pm 0.11 \text{ mg/100g}$. $2.30 \pm 0.11 \text{ mg/100g}$ was reported by Gupta *et al.*, 2013 for *E. officinalis*, *Acaciacatechu* and *Acaciaconcina* and *Hyptisverticillata* by Egbunget *et al.*, 2017 respectively. Tannin has been reported for its astringent, antimicrobial and quick wound-healing properties. It is a heterogeneous group of high molecular weight polyphenolic compounds used for the treatment of hemostasis and hemorrhoids in man.

Table 3: Results of Qualitative Phytochemical Constituents of *Brassica oleracea* Leaf Concentrates

Parameters	Value
Saponin	+
Alkaloids	+
Flavonoids	+
Tannin	+
Coumarin	+
Steroid	+
Terpenoid	+
Cardial glycosides	+
Glycosides	-
Quinones	+
Anthocyanin	-
Phytoseroids	+
Phenols	+

Table 4: Results of Quantitative Phytochemical Constituents of *Brassica oleracea* Leaf Concentrates

Phytochemical Parameters	Value (mg/100g)
Saponin	5.76±0.07
Alkaloids	7.61±0.16
Flavonoids	35.98±0.11
Phenols	21.41±0.06
Tannin	9.84±0.03
Oxalate	8.47±0.42
Phytate	13.22±0.76

This suggests the reason why the sample leaf is used in the treatment of hemorrhoids (Sharma *et al.*, 2019). The level of Alkaloids present in the leaf protein concentrates is 7.61±0.16mg/100g. The alkaloid levels are higher than 4.40±0.01mg/100g reported for the leaf of fluted pumpkin by Ladiet *et al.*, 2016. Alkaloid is one of the bioactive compounds in plants that exhibit therapeutic analgesic, antiplasmodial and bactericidal properties (Ahmed *et al.*, 2013). The value of alkaloids present in the leaf protein concentrates of this sample suggests the reasons for the analgesic properties and antimicrobial properties reported for the leaf of this plant.

The flavonoid value of the *Brassicaoleracealeaf* concentrate is 35.98±0.11mg/100g. The value is higher than 0.36±0.02mg/100g, 0.95±0.04mg/100g and 0.75±0.02mg/100g reported for the leaf, stem and root of *Maesobotryabarteri* respectively by Etukudo and Osim, 2018. The value is also high compared with 1.34±0.02mg/100g and 1.21±0.14mg/100g reported for *Urenalobata* stem and leaves respectively by Abi and Omuha 2014 and Njoku *et al.*, 2020. Flavonoids exhibit antioxidant properties that assist in the prevention of various types of Cancer and tumour. It also inhibits cancer growth, protects against platelet aggregation of microbes, and ulcers and boosts the production of detoxifying enzymes in the body (Okwu and Ndu, 2006). The levels of flavonoids present in the leaf protein concentrates of *Brassicaoleracea* could assist in the roles of flavonoids listed above.

Leaf concentrates of *Brassicaoleracea* contain 21.41±0.06mg/100g of phenols. The value of phenol present in this sample is higher than 0.04±0.01mg/100g and 0.05±0.01mg/100g reported for the leaf and root of *Carpolobialutea* by Olayinka *et al.*, 2019. The value is also high compared to 13.20±0.40mg/100g reported by Egbunget *et al.*, 2014 for *Hyptisvertialata*. Phenols have been reported to exhibit anti-oxidation hepatic toxicity, platelet aggregation, and inhibition of virus and tumour growth in the human body (Asuket *et al.*, 2015). It is also known to remediate inflammation of tissue, ulcers and radical scavenging activities. The leaf protein concentrates of *Brassicaoleracea* can be used

to combat health issues that dietary phenols could treat due to the high amount of phenols present in the sample.

The tannin content of the leaf concentrates of *Brassicaoleraceais* $9.84\pm 0.03\text{mg}/100\text{g}$. The value of tannin present in this sample is higher than $4.89\pm 0.13\text{mg}/100\text{g}$ reported for *Hyptisverticillata* leaves by Egbunget *et al.*, 2017 but low compared to $12.82\pm 0.14\text{mg}/100\text{g}$ and $11.34\pm 0.48\text{mg}/100\text{g}$ reported for round and oval varieties of *Solanummelongena* respectively by Agoreyoet *et al.*, 2012. Tannin assists in the treatment of wounds through direct application in the form of protein precipitation. It is also useful for the treatment of hemostasis and haemorrhoids in men. Tannin has astringent and antimicrobial properties and prevents urinary tract infections.

The presence of a valuable amount of tannin in the leaf concentrate of *Brassicaoleracescan* make the sample useful in the treatment of haemorrhoids, diarrhea fast healing of wounds and bruises (Kumara and Jain 2015). The value of oxalate present in the leaf concentrate of *Brassicaoleracea* is $8.47\pm 0.42\text{mg}/100\text{g}$. The level of oxalate in this sample is lower than $41.72\pm 0.60\text{mg}/100\text{g}$ and $23.97\pm 0.40\text{mg}/100\text{g}$ reported by Agoreyoet *et al.*, 2012 for two varieties of *Solanummelongena* oxalate is one of the phytochemical compounds regarded as antinutrients due to their high tendency to bind metals especially magnesium, calcium and iron to form insoluble salts making them unavailable for the body. (Abdoulayeet *et al.*, 2011). The quantity of oxalate that is considered harmful in food is any dosage that exceeds 2.5g (Idriset *et al.*, 2019). This makes the leaf concentrates of *Brassicaoleraceato* be pharmacologically relevant. Oxalate however assists in the regulation of heavy metal levels in the human body.

The value of phytate present in the leaf concentrate of *Brassicaoleracea* is $13.22\pm 0.76\text{mg}/100\text{g}$. thephytate content of the leaf concentrates of *Brassicaoleraceais* lower than $0.062\pm 0.03\text{mg}/100\text{g}$, $0.025\pm 0.01\text{mg}/100\text{g}$ and $0.019\pm 0.02\text{mg}/100\text{g}$ reported for the leaf root and stem of *Maesobotryabarteri* by Etukudo and Osim 2018. Too much phytate, like oxalate in any food ingredient is dangerous; this is because it has been established to have adverse effects on the absorption and digestion of some mineral elements. It also depletesthe utilization of protein and lipids in the body if it exceeds a minimal lethal dose of 5g for an adult (Kumar *et al.*, 2010 and Morale *et al.*, 2014). The amount of phytate present in the leaf concentrate of *Brassicaoleraceais* lower than the minimal lethal dose, indicating the safe use of the sample against the toxicity of phytic acid that would lead to blockage of the kidney tubule. Phytate however presents metal poisoning and exhibits anti-oxidant properties which prevent tumour growth (Alkarawi and Zotz 2014).

The results of antimicrobial susceptibility showed that only six (6) out of the nine (9) microorganisms tested with the crude extracts of the selected sample's leaf protein concentrate were susceptible to the extracts while three microorganisms showed resistance to the ethanolic extracts of the sample. All the microorganisms were, however, susceptible to Ampicillin, though at variable concentration ranges (6-30µg/ml). The results of antimicrobial analysis of the leaf protein concentrates of *Brassica Oleracea* showed that the antimicrobial activity of the plant was not altered by the processing effect, though the concentration required to combat the selected microorganism was higher than the standard drug.

Table 5: Antimicrobial activity of the ethanolic and extract from Brassica Oleracea

Microorganism	The activity of the Extracts
<i>Staphylococcus aureaus</i>	+
<i>Streptococeuspyogenes</i>	+
<i>Streptococcus faecaias</i>	-
<i>Pseudomonas aeruginosa</i>	-
<i>Klebsiella pneumonia</i>	-
<i>Proteins, mirabites</i>	+
<i>Candida kruisei</i>	+
<i>Candida albicans</i>	+
<i>Candida valida</i>	+
<i>Staphylococcus aureaus</i>	+

Antimicrobial activity ≥ 7 mm

Table 6: Minimum Inhibitory Concentration of the Plant Extracts

Bacteria	Plant extract µg/ml	Amplicin Standard drug µg/ml
<i>Proteus mirabilis</i>	70	30
<i>Staphylococcus aureaus</i>	60	40
<i>Streptococeuspyogenes</i>	20	15
<i>Streptococcus faecaias</i>	50	35
<i>Pseudomonas aeruginosa</i>	10	8
<i>Klebsiella pneumonia</i>	10	6

Conclusion

The result of the present investigation on the *Brassicaoleracealeaf* protein concentrates reveals the presence of valuable dietary constituents, mineral elements and phytochemical compounds. This

implies that the leaf protein concentrate of the sample would be useful as food additives, and dietary supplements and could be used as raw materials for pharmaceutical products due to the presence of phytochemical compounds of strong bioactive tendency properties.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. No Artificial Intelligence was used
- 2.
- 3.

REFERENCES

- Abdoulaye, C., Brou, K., Jie, C. (2011). Phytic acid in cereal grains; structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *Am. J. plant nutr. Fertn. Tecn.*1(1): 1-22
- Abi, T. A. & Onuha, E. N. (2014). A preliminary investigation into the phytochemicals, vitamins and mineral constituents of the leaf of two tradomedicinal plants-Urenalobata and Cassia alata used in Nigeria. *Journal of Applied Chemistry* 72(1), 01-04.
- Adom, K. K., Sorrell, M. E. & Liu, R. H. (2015). Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *Journal of Agriculture and food Chemistry* 53, 2297-2306.
- Agoreyo, B.O. Obansa E.S. and Obanor E.O. (2012). comparative nutritional and phytochemical analysis of two varieties of Solanummelongena. *Science world Journal* 7 (1): 5-5.
- Ahmed, A. M., Lydia, J. & Campell, J. L. (2012). Evaluation of backing properties and sensory quality of wheat-cowpea flour. *World Academy of Science, Engineering and Technology* 70, 2012

- Ajani, O. A., Owoeye, T. F., Olasehinde, G. I., Akinlabu, D. K., Owolabi, E. F. and Audu, O. Y. (2016). Characterization, proximate composition and Evaluation of Antimicrobial activity of seed oil of *Bauhinia tomentosa*. *Journal of Biological Sciences* **16**(4):102-111.
- Aletan U.I. and Kwazo H.A. (2019). Analysis of the Proximate Composition, Anti-Nutrients and Mineral Content of *Maerua Crassifolia* Leaves, *Nigerian Journal of Basic and Applied Science*, **27**(1): 89-96
- Alkarawi, H. H.; Zotz, G. (2014). Phytic acid in green leaves of herbaceous plant –temporal variation in situ and response to different nitrogen phosphorus fertilizing regimes *A. B. plant* **6**, 1-7.
- Anwani, S. E.; Etsuyankpa, M. B.; Ogah, S. P. I. (2020). Assessment of the proximate and elemental composition of the leaves, stem bark and roots of *bobgunnia fistuloides*. *World Journal of Applied Chemistry* **5**(4): 57-64.
- Anyasor, G. N.; Onajobi, F. D.; Osilesi, O. and Adebawo, O. (2014). Proximate composition, mineral content and in vitro antioxidant activity of leaf and stem of costus afer (Gingerlily). *Journal of International Ethnopharmacology* **16**, 24-30.
- AOAC International; (2016). Official Methods of Analysis of AOAC International. 20th ed. Gaithersburg, MD, USA: p. 3172.
- Asuk, A. A.; Agiang, M. A.; Dasofunjo, K.; Willie, A. J. (2015). The biomedical significance of the phytochemical, proximate and mineral compositions of the leaf, stem bark and root of *Jatropha curcas*. *Journal of Tropical Biomedicine* **5**(8): 650-657.
- Awol, A. (2014). Phytochemical screening, proximate and mineral composition of sweet potato leaves grown in Tepi provision. *South-West of Ethiopia, Science Technology and Arts Research Journal* **3**(3): 112-115
- Ayanda, I. O.; Dedeke, G. A.; Ekhator, I. U. and Etiebet, M. K. (2018). Proximate composition and heavy metal analysis of three aquatic foods in makoko river Lagos, Nigeria. *Journal of Food quality* **7**, 6.
- Bakari S.; Daoud, A.; Smaoul, S.; Gharsalah, N. and Kadri, A. (2017). Proximate analysis, mineral composition, phytochemical contents, antioxidant and antimicrobial activities and GC-MS investigation of various solvent extract of *Cactus cladode*. *Journal of Food Science and Technology* **37**, 2.
- Beshaw, T., Demssie, K., Tefera, M. and Guadie, A (2022) Determination of proximate composition, selected essential and heavy metals in sesame seeds (*Sesamum indicum* L.) from the Ethiopian markets and assessment of the associated health risks, *Toxicology Reports*, **9**, 1806-1812.
- Chuck C. N. and Ugochi F. H. (2012). Absorption of iron from ferritin is independent of heme iron and ferrous salts in women and rat intestinal segments. *J Nutr.* **142**:478–83.
- Deshmukh B.S. and Waghmode, A. Role of wild edible fruits as a food resource: Traditional knowledge. *Int. J of Pharm. & Life Sci.* **2011**; **2**(7):0976-7126.

- Egbung, G. E., Anosike, C., Boniface, A. U., Ogar, I. and Nna, V. U. (2017). Phytochemical evaluation and GC-MS analysis of Hyptisverticillation cultivated in Calabar cross river state. *Nigeria Journal of Biology, Chemistry Science* **11**(5): 2548-2559.
- Etukudo, M. M. & Osim, S. E. (2018). Assessment Of Mineral, Proximate And Phytochemical Composition of Leaf, Stem and Root of Maesobotryabarteri (BAILL) from Secondary Forest in Akwalbom State. *International Journal of Advanced Research*. 6. 500-505.
- Ferdaus, M. D. J.; Ferdous, Z.; Sara, R. J.; Mahin, M. D. G. and Faruque, M. D. O. (2020). Total antioxidants activity and proximate analysis of selected fruits and vegetables in Jashore Region, Bangladesh; *Current Research in Nutrition and Food Science*. **8**(3): 785-797.
- Gebre, A. and Chandravanshi B. S. (2012). Levels of essential and non-essential metals in *Rhamnus prinoides* (GESHO) cultivated in Ethiopia, *Bull. Chem. Soc. Ethiop.*, 26 (3): 329-342.
- Gundersen, E., Christiansen, A. H.C., Jørgensen, K. and Lübeck, M. (2022). Production of leaf protein concentrates from cassava: Protein distribution and anti-nutritional factors in biorefining fractions, *Journal of Cleaner Production*, 379, 1, 134 -140.
- Gupta, M., Thakur, S., Sharma, A. and Gupta S. (2013). Qualitative and Quantitative Analysis of Phytochemicals and Pharmacological Value of some Dye Yielding Medicinal Plants. **29** (2): 475-481.
- Idris, O. A.; Wintola, O. A. and Afolayan, A. T. (2019). Comparison of the proximate composition, vitamins (Ascorbic acid & Tocopherol and Retinol), Anti-Nutrients (Phytate and oxalate) and the GC-MS Analysis of the Essential oil of the Root and leaf of *Rumex crispus* L. *Journal of Mdpi.com* 1: 8.
- Kassegn, H. H. (2018). Determination of proximate composition and bioactive compounds of the Abyssinian purple wheat. *Cogent Food & Agriculture* **4** (1): 1-32
- Kumar, V.; Sinha, A. K.; Makkar, H. P. S.; Becker, K. (2010). Dietary roles of phytase in human nutrition: A review: *Food Chem.* **120**: 945-959.
- Kumara, M. and Jain, S. (2015). Tannins, an anti-nutrient with positive effect to manage diabetes. *Research Journal of Recent Science* **1**(12): 70-73.
- Koubova, J., Menke, D. B., Zhou, Q., Capel, B., Griswold, M. D., and Page, D. C. (2006). Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc. Natl. Acad. Sci. USA* **103**, 2474–2479.
- Ladi O. J., Ojo O. C., Awodi Y. P. and Alfa I. N. (2016). Proximate composition, mineral and phytochemical contents of some leafy vegetables native to Igala kingdom Kogi State Nigeria. *International Journal of Biochemistry Research & Review*. **15** (4): 1-11.
- Morales P., Ferreira I. C. F., Carvalho A. M., Sanchez-mata M. C., Camara M., Fernandez-Ruiz V., Pardo-de-santayana M., Tardio, J., Mediterranean M. (2014). Non-cultivated vegetables as dietary sources of compound with antioxidant and biological activity. *LWT food sci. technol.* **55**: 389-396.
- Murugesan, M., Myneni, V., Mengistu, Y., and Kandasamy, K. (2021). Evaluation of anti-cancer

activity of phytosomes formulated from aloe vera extract. *Materials Today: Proceedings*. 42. 10.1016/j.matpr.2020.11.047.

- Ngaha, N. M. I.; Dahlan, I.; Massoma, L. D.; Mandengue, S. H. and Yusuff, A. A. (2016). Comparative Proximate Analysis of Leaves and Bark of *Alchornea Cordifolia* (Euphorbiaceae). *Journal of Agriculture and Environmental Sciences* **5**(1): 84-90.
- Njoku C. E., Alaneme K. K., Omotoyinbo J. A., Ekeleme A. C., Ugwu E. I. and Ikele U. S. (2020). Phytochemical, proximate and Mineral Analysis of *Urenalobata* stems from Imo State Nigeria. *Journal of International Conference on Engineering for Sustainable World*.
- NRC (1989) National Research Council Recommended Daily Allowance, National Academy Press Washington D.C. Pp 42.
- Ogbemudia, R. E., Chika, N. B. and Benedicta, A. (2018). Mineral and proximate composition of soya bean, *Asian Journal of Physical and Chemical Science*, vol. 4, no. 3, pp. 1-6,
- Okwu, D. E. and Udu, C. U. (2006). Evaluation of the phytonutrients, mineral and vitamin contents of some varieties of yam. *International Journal of mol. Med. and Advance sci.* **12**(2): 199-203.
- Olayinka B. U., Ogungbemi R. F., Abinde O. O., Lawal A. R., Abdulrahman, A. A. and Efejere, E. O. (2019). Proximate and phytochemical compositions of leaf and root of (Cattle stick) *carpolobialutea* G. Don. **23**(1): 53-57.
- Ooi D. T., Igbal S. and Ismail M. (2012). Proximate composition, Nutritional Attributes and Mineral composition of *Peperomia pellucida* L. (Ketumpangan Air) Grown in Malaysia; *Mdpi Journal Molecules* **17**: 1139-1145.
- Paul S. H., Usman A. A., Gana I. N., Manase A., Adeniyi O. D. and Olutoye M. A. (2018). Comparative study of Mineral and Nutritional Composition of a Multifunctional Flora Composite Formulated from Seven Medicinal Plants and their Applications to Human Health. *Engineering technology open access Journal* **1**(5): 34-40
- Prasad, A., Mills, A., Prasad, and Albert J. (2010) Critical Management Studies and Business Ethics: A Synthesis and Three Research Trajectories for the Coming Decade, *Journal of Business Ethics* **9**/Supplement 2, pp.227-237.
- Prasad, K. (2017). HPLC Analysis of Amino Acid and Antioxidant Composition of Three Medicinal Plants of (Pithoragarh) Uttarakhand Himalayas. *Journal of Analytical and Pharmaceutical Research* **6** (5): 38-42
- Schaus, D. and Sullivan, R. (2015). Extended Spectrum Beta- Lactamases: A Minireview of Clinical Relevant Groups. *Journal of Medical Microbiology & Diagnosis*. **4**, 4.203-208.
- Suleiman M., Safiya M. A., Nasiru Y., Alhassan M. and Bello H. J. (2019). Proximate Composition and Mineral Analysis of *Bryocarpus Coccineus*; *Chem Search Journal* **10**(1): 33-37.

- Soetan K. O. Olaiya C. O. and Oyewole O. E. (2010).The importance of mineral elements for humans, domestic animals and plants. A review; *Afr. J. Food science* 4:200-222.
- Sharma P., Shri R. Ntie-Kang, F. and Eboatu A. N. (2019).Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria.247-270.
- Velmurugan, G. and ParvathiAnand, S., 2017. Antifungal activity and quantitative phytochemical analysis of *Phyllodiumpulchellum* L. Desv.- An important medicinal plant *Int.J.Curr.Res.Biosci.Plantbiol.* 4(8): 67-72. doi: <https://doi.org/10.20546/ijcrbp.2017.408.009>