Assessment of Proximate, Mineral, Phytochemical and Antimicrobial Properties of *Brassica oleraceaeL*. Leaf Protein Concentrates

ABSTRACT

Food insecurity and malnutrition have led tothe search for alternative and affordable measures to address food shortage problems and associated health issues worldwide. This brings 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 beforebeing processed into the leaf protein 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. The sample extracts showed antimicrobial potential against the ten selected microbes.

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

INTRODUCTION

Plants and plant products have been used for many decades as remedies for different purposes. 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 (Velmurugan and Anand, 2017).

However, due to the gradual decline in protein supply from animal sources, calorie malnutrition and widespread deficiency diseases due to food shortage coupled with the global increase in inflation, the focus of nutritionists has drifted toward protein from plant and medicinal 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 protein concentrates of some green vegetables (Deshmukh and Waghmode 2011) cassava leaves (Gundersen *etal.*, 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. It is therefore the objective of this present study is to evaluate the nutrient composition phytochemical constituents, mineral composition and antioxidant potential of *Brassica oleracea L*leaf protein concentrates.

Brassica oleracea L 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 found growing on rocky sea cliffs along the coastline leaf is eaten as a vegetable throughout the world, after 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. The seeds of the plants are considered to be laxative, diuretic stomachic and antihelmintic. Therefore the various claims in the existing literature on the usefulness of *Brassica oleraceaL*. leaf as a nutraceutical plant could be investigated in the leaf protein 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 specializes in planting the plant on a commercial scale before the 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 protein concentrates.

Sample Preparation

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

Proximate Analysis

Proximate contents of thedried, powdered leaf ofthe selected plant samples were determined using the variousmodified methodsofthe 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

TheAOAC(2016)formalanalysismethodwasusedtodeterminemoisturecontent**.** 2 g of each of the selected samples was weighed into three clean, dried, and pre-weighed porcelain crucibles. The leaf protein concentrate of *Brassicaoleracea* was dried in the moisture extraction oven at 100 °C for 3 hours. Thedriedsampleswereremovedfromtheoven,cooledinadesiccatorandreweighed**...** The last procedure was repeated several times 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:

Moisture (%) = $\frac{W2-W3}{W2-W1}$

Where W_1 = Weight of empty moisture can

W_2 = Weight of empty moistureCan 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 protein concentrates of *Brassica oleracea*. 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:

The crude fibre was determined as $\frac{W2-W1}{Weight\ of\ the\ sample}\ x\ 100$

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

W_3 = Weight of crucible + ash of the sample

Determination of crude protein

The Kjeldahl method was used to determine the crude protein constituents of *Brassicaoleracea*. A 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_4were 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:

$$N_2(\%) = \frac{100 \times M \times 14 \times Vt}{W \times 100 \times Va} \times T \times B$$

Where W = Wt of sample, M = Molarity of titrant (0.02m)

Vt = Total digest volume (100ml) Va = Volume of digest analysed

T = Sample titre value B = Blank titre value

Determination of Fat content

4 g of the leaf protein 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 desiccatorsand reweighed. The fat content was calculated as follows:

$$\text{The weight of the fat extracted} = \frac{W2 - W1}{\text{Weight of the sample}} \; x \; 100$$

Where W_1 = Weight (g) of the empty extraction flask

W_2 = Weight of flask + 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:

\% Carbohydrate = 100 - (% Crude protein + % Crude fat + %crude fibre + % Ash + % Moisture)

Determination of Energy Value of Sample

The gross energy of the leaf protein concentrate of *Brassica oleracea* was calculated using the formula:

The grass energy value = (% Crude protein x 4) + (% Crude fat x 9) + (% Carbohydrate x 4)

Determination of Mineral Composition

The dried leaf protein 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 HNO_3 and 4.5 mL $HClO_4$. (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 370°C for 24 hours. After this period it is 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 80-2% Tween. 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 protein concentrates of *Brassica Oleracea* are *Staphylococcus aureaus, Streptococeus pyogenes, Streptococcus faecaias, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteins, mirabites, Candida kruisei, Candida albicans* and *Candida valida.* Which were grown in a nutrient medium for 6 hours. Then, 100 µL of 106 cells/mL was injected into tubes containing nutrient solution supplemented with various concentrations (8 – 70µL) of extracts and Standard Drug (Ampicilin). After 24 hours at 370°C, the MIC of each sample was determined by measuring the optical density in a spectrophotometer (620 nm) and comparing the sample readings to the non-inoculated nutrient medium.

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

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

The results of proximate constituents of *Brassica oleracea* leaf protein concentrates are shown in Table 1. The moisture content of the sample is 8.80±0.16g/100g. the percentage moisture composition of this sample is in the same range as the values reported for the lead and root of *Rumex cripsus (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 (Adom *et 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 protein concentrates of *Brassica oleracea* contain 1.38±0.10g/100g of Ash. The ash content of this sample is slightly high compared with 0.54±0.08g/100g and 0.45±0.04g/100g reported for bottle and pointed gourd respectively by Ferdaus *et 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±0.47g/100g reported for *Maervacrassifolia* leaves by Aletan and Kwazo 2019.

The crude fat content of *Brassica oleracea* leaf protein concentrates is 4.66±0.12g/100g. The crude fat of this sample is higher than 2.28±0.09g/100g reported for seed oil of *Bouhinia tomentosa* by Ajani *et al.,* 2016. The value is however low compared with 28.2±0.00g/100g 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 protein concentrates of *Brassica oleracea* is 35.52±0.31g/100g. The crude protein values of this sample are higher than 3.35±0.22g/100g reported for *Bryocarpuscoccineus* and 10.36±0.07g/100g reported for *Peperomiapellucid* by (Suleiman *et al.,* 2019) Ooi *et 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 leaf protein content of *Brassica oleracea* is 3.16±0.06g/100g of crude fibre. The value of crude fibre in this sample is low compared with 6.5g/100g and 8.5g/100g reported for white and purple wheat by Kassegn (2018) and 28.00±0.69g/100g 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 health and proper functioning (Kassegn 2018 and Ahmed 2014). A high amount of *Brassica oleracea* leaf protein concentratewill be consumed to meet to required dietary fibre.

The leaf protein concentrates of *Brassica oleracea* contain 46.45±0.23g/100g of nitrogen-free extractive. The value of crude carbohydrate in this sample is higher than 42.50g/100g reported for *Cactuscladode* by Bakari *et al.,* (2017) but lower than 74.22g/100g reported for wheat by Ahmed *et al.,* 2012. Carbohydrate in food is the major source of energy in food, they also help to regulate nerve tissue at high concentration. 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 mg/100g	35.81 ±0.23	3.37± 0.01	24.71± 0.02	0.24± 0.00	0.91± 0.00	0.90± 0.01	0.50± 0.00	0.10± 0.00	0.00 ±0.0	0.00 ±0.0	0.03± 0.00	0.00± 0.00	0.02 ±0.01

The concentration of different minerals present in the leaf protein concentrates of *Brassica oleracea* is presented in Table 2. The sample had 35.81±0.23mg/100g of sodium, the values are low compared with 84.11mg/100g and 50.55mg/100g reported for turmeric and scent leaves respectively by Paul *etal.*, 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 Murugese*t.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 protein 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 *Maesobotryabarteri* 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*etal.*, 2020). The recommended daily allowance of dietary potassium is 200mg per day for an adult male. However large quantity of *Brassicaoleracea* will be required to meet the recommended daily allowance.

The concentration of calcium in the leaf, protein concentrates of *Brassica oleracea* 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*etal.*,2014). The value of calcium in the *Brassica oleracea* leaf protein concentrates is higher than 6.77±0.21mg/100g and 8.43±0.36mg/100g reported for the leaf and root of *Maesobotryabariteri* byEtukudo 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 protein 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 *Maesobotryabarteri* 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 (Soetan*et al.,* 2010).

The concentration of phosphorus present in the leaf concentrates of *Brassica oleracea* is 0.91±0.00mg/100g. The value of phosphorus in this sample is higher than 0.04±0.01mg/100g and 0.03±0.01mg/100g 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±0.23mg/100g and 1.33±0.12mg/100g reported for the leaf and root of *Jatrophacurcas* respectively by Asuk*et 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 (Anyasor*et 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 *Brassica oleracea* 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 heamoglobin in the humanbody, 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 *Brassica oleracea* is 0.90±0.01mg/100g. The value is too low compared to the 9.35mg/100g reported for *Urena lobata* 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 protein concentrates of *Brassica oleracea* is 0.50±0.00mg/100g while the value of chromium is 0.10±0.01mg/100g. The value of the zinc in this sample is in the range of 0.43±0.10mg/100g 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±0.00mg/100g and 0.02±0.00mg/100g reported for the leaf and root of *Jathropacurcas* byAsuk*et 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 protein concentrates of *Brassica oleracea* while lead and copper were present in small concentrations of 0.03±0.00mg/100g and 0.02± 0.01mg/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±0.07mg/100g. The value of saponin in this sample is higher than 1.10±0.05mg/100g, and 2.10±0.11mg/100g. 2.30±0.11mg/100g was reported by Gupta *et al.*, 2013 for E. officinalis, *Acaciacatechu* and *Acaciaconcina* and *Hyptisverticillata* by Egbung*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: 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: **Quantitative Phytochemical Constituents of *Brassica oleracea* Leaf Concentrates**

Phytochemical Parameters	Value
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 Ladi*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 *Brassica oleracea* 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* stemand 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 *Brassica oleracea* could assist in the roles of flavonoids listed above.

Leaf protein concentrates of *Brassica oleracea* 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 Egbung*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 (Asuk*et al.,* 2015). It is also known to remediate inflammation of tissue, ulcers and radical scavenging activities. The leaf protein concentrates of *Brassica oleracea*

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 protein concentrates of *Brassica oleracea* is 9.84±0.03mg/100g. The value of tannin present in this sample is higher than 4.89±0.13mg/100g reported for *Hyptisverticillata* leaves by Egbung*et al.,* 2017 but low compared to 12.82±0.14mg/100g and 11.34±048mg/100g reported for round and oval varieties of *Solanummelongena* respectively by Agoreyo*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 heamorrhoids in men. Tannin has astringent and antimicrobial properties and prevents urinary tract infections.

The presence of a valuable amount of tannin in the leaf protein concentrate of *Brassicaoleraces*can make the sample useful in the treatment of haemorrhoids, diarrhea fast healing of wounds and braises (Kumara and Jain 2015). The value of oxalate present in the leaf protein concentrate of *Brassicaoleracea* is 8.47±0.42mg/100g. The level of oxalate in this sample is lower than 41.72±0.60mg/100g and 23.97±0.40mg/100g reported by Agoreyo*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. (Abdoulaye *et al.,* 2011). The quantity of oxalate that is considered harmful in food is any dosage that exceeds 2.5g (Idris *et al.,* 2019). This makes the leaf protein concentrates of *Brassica oleracea* to 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 protein concentrate of *Brassica oleracea is 13.22*±0.76mg/100g. the phytate content of the leaf protein concentrates of *Brassica oleracea* is lower than 0.062±0.03mg/100g, 0.025±0.01mg/100g and 0.019±0.02mg/100g 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 protein concentrate of *Brassica oleracea* is 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
Staphylococcus aureaus	+
Streptococeus pyogenes	+
Streptococcus faecaias	-
Pseudomonas aeruginosa	-
Klebsiella pneumonia	-
Proteins, mirabites	+
Candida kruisei	+
Candida albicans	+
Candida valida	+
Staphylococcus aureaus	+

Antimicrobias activity ≥ 7mm

Table 6: Minimum Inhibitory Concentration of the Plant Extracts

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

Conclusion

The result of the present investigation on the *Brassica oleracea* leaf 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.

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