







## Phytochemicals, proximate and elemental analysis, antioxidant and cytotoxic potentials of purple (*Capitata F. rubra*) and green (*Capitata Linn.*) *Brassica oleraceae* (cabbage)

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### Abstract

Malnutrition remains a significant impediment to growth in many countries. Exploring the medicinal potentials of exotic vegetables represents a strategic approach to achieving sustainable development goals. This study focused on the evaluation of proximate, elemental, and phytochemical composition, including the antioxidant and cytotoxic potentials of green and purple cabbage varieties. The findings revealed different percentages of moisture, crude fat, fibre, protein, and carbohydrates in both varieties. Calcium, magnesium, potassium, sodium, iron, copper, zinc, saponins, terpenoids, flavonoids, phenolics, tannins, anthraquinones, and steroids were also detected and quantified. The green (IC<sub>50</sub>=186.3 µg/mL) and purple (IC<sub>50</sub>=187.6 µg/mL) cabbage extracts demonstrated broad-spectrum 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, as compared with the standard, ascorbic acid (IC<sub>50</sub>=267 µg/mL). Similarly, the green (IC<sub>50</sub>=148.7 µg/mL) and purple (IC<sub>50</sub>=103.9 µg/mL) cabbage extracts exhibited nitric oxide inhibitory activity, as compared with the standard, ascorbic acid (IC<sub>50</sub>=135.2 µg/mL). The green (CC<sub>50</sub>=10.6 µg/mL) and purple (CC<sub>50</sub>=16.66 µg/mL) cabbage extracts exhibited an inhibition on the growth of Rhabdomyosarcoma cell lines as compared with the standard, Vincristine (CC<sub>50</sub>=0.30 µg/mL). These results support the utilization of the two cabbage varieties as dietary supplements, potentially aiding in the discovery of anticancer drugs and the management of other disease conditions owing to their antioxidant properties.

**Keywords:** Antioxidant; Carbohydrates; Calcium; Malnutrition; Phenolics; Rhabdomyosarcoma

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## INTRODUCTION

In many countries around the world, particularly in developing regions, malnutrition continues to affect the growth of individuals. Globally in 2022, 149 million children under 5 were estimated to be stunted (too short for age), 45 million were estimated to be wasted (too thin for height), and 37 million were overweight or living with obesity, frequently as a result of environmental deterioration, drought, and biodiversity loss [1]. A rising trend of malnutrition and acute food insecurity is evident across almost all African nations, including Nigeria. The persistent issue of undernourishment in Africa has profound consequences for its population, stemming from both the inadequate quality and quantity of food consumed by Africans over an extended period [2,3]. According to the fifth wave of the Multiple Indicator Cluster Survey (MICS5), malnutrition affects children under the age of five more frequently in rural than in urban regions in Nigeria [4]. Making quality food accessible and affordable for everyone in the nation is necessary to achieve food security, improve nutrition, ensure healthy lives and promote well-being for all ages [5]. A disturbing percentage of Africans face challenges in accessing and acquiring sufficient knowledge about high-quality food, highlighting the urgent need to address this issue.

Considering their rich content of dietary fibre, vitamins, minerals, electrolytes, and phytochemicals, particularly antioxidants, vegetables should be prioritized when providing dietary recommendations [6]. Inadequate vegetable consumption has been associated with the development of chronic diseases, including many malignancies, cardiovascular diseases, high blood pressure, hypercholesterolemia, osteoporosis, chronic obstructive pulmonary diseases, respiratory issues, mental health, and other ailments [7]. Despite the increasing recognition of the health benefits of vegetables, both children and adults

tend to consume less than the recommended amounts [8,9]. Consequently, prioritizing efforts to raise awareness about the importance of vegetable consumption is crucial, particularly in Africa where nutritional-associated health challenges have seen a significant rise on a global scale.

Cruciferous vegetables, also known as vegetables of the Brassicaceae family, encompass a diverse group of plants. According to Higdon et al. [10], popular examples of cruciferous vegetables include broccoli, Brussels sprouts, kale, mustard, cabbage, turnips, cauliflower, bok choy, and Chinese cabbage. Widely grown worldwide, cruciferous vegetables are highly regarded because of their nutritional value, serving as good sources of soluble fibre, vitamin C, and a variety of other nutrients and phytochemicals. Recent studies highlight their abundance in carotenoids, tocopherols, and ascorbic acid, serving as natural antioxidants with potential to scavenge free radicals and shield us from their damaging effects [11].

Cabbage (*Brassica oleraceae* Linn., Brassicaceae) stands out as one of the most globally consumed vegetable due to its adaptability to a variety of climatic situations and soil types, ease of production and storage, and high nutritional value [12]. These vegetables, classified as exotic since they are not native to Nigeria, typically flourish in temperate zones worldwide. Cabbage cultivation is particularly successful in some few local government areas of Plateau State [12]. Due to its unique characteristics, cabbage has been used in traditional medicine mostly for rheumatic pain, lymphatic artery and vein irritation, bruising, sprains, mastitis, peptic ulcer, various types of cancer, digestive issues, and gout as well as for detoxification purposes [11,13]. Its therapeutic potential extends to both internal and external ailments, reflecting its broad spectrum of uses. Consequently, a comparative assessment of the phytochemicals, proximate and elemental

composition, as well as antioxidant and cytotoxic potentials of green (*Capitata L.*) and purple (*Capitata F. rubra*) cabbage was conducted.

## EXPERIMENTAL METHODS

**Collection of plants and extraction.** Samples of green and purple cabbage were collected at a farm in Kwall village of Plateau State's Bassa local government area. The green and red cabbage leaves were separately cut into pieces, air-dried, and subsequently ground into powder, all conducted at room temperature. The plant materials were then separately subjected to maceration in hydro-methanol (30:70) for 72 hours [14], with intermittent agitation using 200 g plant material, respectively. Following maceration, the extracts were filtered and subsequently concentrated under vacuum. Once dried, the extracts were stored at 4°C until required for further analysis.

**Proximate analysis.** The Association of Official Analytical Chemists (AOAC) [15] method was used to measure the samples' moisture, ash, crude fats, fibres, proteins, and carbohydrates. The weight difference method was used to calculate the ash and moisture content. Using the Soxhlet system and petroleum ether (40 to 60°C) for eight hours, crude fat was extracted. The defatted samples were successively digested with 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% sodium hydroxide solutions to remove crude fibres.

The micro Kjeldahl method, which Pearson [16] characterized as including digestion, distillation, and ultimately titration of the sample, was used to assess the nitrogen content value, which is the precursor for the protein content of a substance. Protein content was estimated by multiplying the nitrogen content value by a factor of 6.25. The total carbohydrate was calculated by difference method and the nitrogen-free extract (NFE), calculated as % NFE = 100 - % (a + b + c + d

+ e) where a = protein, b = fat, c = fibre, d = ash, e = moisture [16,17]. All the proximate values were reported in % [15,18]. The proximate analyses were done in triplicates.

**Analysis of elements.** The method developed by AOAC [15] was used to determine the mineral content. 2 g of the powdered plant samples were put in a crucible and heated to 550°C for 6 h in a muffle furnace. The resultant ash was heated slowly for 20 minutes while being dissolved in 10 mL of 10% HNO<sub>3</sub>. It was heated, and filtered, and the quantity of minerals was determined from the filtrate. The atomic absorption spectrophotometer (AAS) was used to measure the quantity of lead (Pb) and cobalt (Co), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), and chromium (Cr) respectively. The quantity of sodium (Na) and potassium (K) were determined using a flame emission spectrophotometer at the appropriate wavelength, temperature, and lamp current for each element. Results were expressed in mg/g.

**Qualitative phytochemical evaluation.** Phytochemical analysis of the extracts was performed using standard qualitative methods [19,20]. The cabbage extracts were analysed for the presence of saponins, alkaloids, flavonoids, tannins, phenolics, coumarin, terpenoids, steroids, anthraquinone, quinone, anthocyanin, cardiac glycosides.

## Quantitative phytochemical evaluation

**Saponin content** Obadoni and Ochuko's [21] approach was employed. One gramme of each powdered sample was macerated into 100 mL of 20% aqueous ethanol in a conical flask and heat at 55°C, over a hot water bath for 4 h while being stirred continuously. Filtering the mixture allowed the residue to be extracted again using 200 mL of 20% ethanol. The filtrate was dried over a water bath at roughly 90°C. The dried extracts were dissolved with water into a 250 mL separatory funnel, 20 mL of diethyl ether was added, and the mixture

was agitated. While the ether layer was discarded, the aqueous layer was recovered. The cleansing procedure was repeated. 60 mL of n-butanol was added. Two separate washes with 10 mL of 5% aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the residual solution was heated. Following evaporation, the samples were dried in an oven to a consistent weight, and the amount of saponin was determined.

**Total phenolic content.** The amount of phenolics in the extracts was measured using Folin-Ciocalteu's phenolic oxidizing reagent, which was described by Singleton et al. [22]. To begin, 0.1 mL of the stock solution in distilled water (0.9 mL) was mixed thoroughly with the phenolic reagent (0.2 mL), and then a 7% Na<sub>2</sub>CO<sub>3</sub> (w/w) solution (1 mL) was added after five minutes, bringing the total volume up to 2.5 mL. The mixture was then incubated at 30 °C for 1½ h. Absorbance at 750 nm was measured against a negative control (1 mL of distilled water with no extract). To calculate the extract's gallic acid equivalent (GAE), a calibration curve was generated using gallic acid at 0.2-1.0 mg/mL as a standard.

**Tannin content.** For the determination of tannins, the Folin-Ciocalteu method was used. To a volumetric flask of 10 mL, containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% Na<sub>2</sub>CO<sub>3</sub> solution, and 0.1 mL of the sample extract, a set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were added. The mixture was diluted with distilled water and shaken well. After 30 minutes at room temperature, the absorbance for test and standard solutions was measured at 725 nm using a UV/Visible spectrophotometer. The tannin content was then expressed in terms of milligrams of GAE per gram of extract.

**Total flavonoid content.** The extracts were analysed for their content of gallic acid equivalents (GAE) per gram. Meanwhile, the

flavonoid content was determined using the standard quercetin with varying concentrations (0.2 to 1 mg/mL). The method used for this test was the aluminium chloride colorimetric test described by Miliauskas et al [23]. In this process, 0.1 mL of the extracts/standard in distilled water (0.4 mL) was mixed with sodium nitrite (5%) (0.1 mL). After five minutes, 10% aluminium chloride (0.1 mL) and sodium hydroxide (0.2 mL) were added, and then distilled water was added to make the final volume 2.5 mL. A blank was used to compare the absorbance at 510 nm. The total flavonoid level of the plant was determined as mg quercetin equivalent/gram of crude extract using this method.

#### **Antioxidant assays**

**DPPH assay.** The antioxidant properties of the extracts were tested using two methods, DPPH and bleaching [24]. To do this, 100 µL of sample dilutions (blank/standards/plant) were added to 150 µL of DPPH (3 g dissolved in 60 mL of methanol) in a 96-well microplate. After 30 minutes of incubation, stable free radicals were formed in the solutions of aqueous or methanol. These free radicals delocalize free electrons, resulting in a purple solution. The concentrations (1000 - 62.5 µg/mL) were calculated at 517 nm in the Spectramax Gemini XS microplate reader. As the value of DPPH at 517 nm decreases, the radical scavenging activity increases [25]. The percentage inhibition was calculated using the formula  $((\text{Blank absorbance} - \text{Sample absorbance}) / (\text{Blank absorbance})) \times 100$ . The graph plotted the percentage inhibition against the concentrations of the extracts to compute the fifty percent inhibition (IC<sub>50</sub>) of the concentration of extracts.

**Nitric oxide scavenging assay.** Minor modifications were made to the Balakrishnan et al. [26] protocols in order to apply them to this quantification. Tubes containing various quantities (ranging from 31.25 to 1000 µg/mL) of extracts were treated with sodium nitroprusside (40 mM) in phosphate buffer

saline (20 mM, pH 7.4) for three hours at 29°C. The control experiment followed the same procedures but used the same amount of buffer and no test chemicals. After three hours, equal parts of the supernatant from the incubated samples and freshly produced Griess reagent were added to 96 micro-well plates. After being incubated for 15 minutes, the colour produced by the nitrite and sulphanilamide diazotization process with naphthyl ethylenediamine hydrochloride sequential coupling was visible. A spectrophotometer was used to measure the absorbance at 550 nm. The test agents were compared to ascorbic acid using the same approach. A calibration curve was created with a 1:50 dilution of 10 mM NaNO<sub>2</sub>. This was done in triplicate. The percentage inhibition of the extracts compared to the negative control was calculated using the formula: Percentage inhibition = (Average of test agents) / (Average of control) × 100.

#### **MTT assay to determine the extracts' effect on cell proliferation**

**Cell culture.** The Rhabdomyosarcoma cells used in this study were obtained from the WHO reference polio laboratory located at the University College Hospital in Ibadan, Nigeria. The cells were cultured in Eagle's minimum essential medium (EMEM) which was supplemented with 10% Foetal Bovine Serum (FBS), 1% non-essential amino acids and vitamin solution, penicillin (100 units/mL), streptomycin (100 mg/mL), L-glutamine (2 mM), and 0.07% NaHCO<sub>3</sub>. The cell cultures were maintained in a humid environment with 5% CO<sub>2</sub> at 37°C and split every two weeks.

**Cytotoxicity assay.** The ability of the cells to cleave to MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Sigma, Chem), via the action of mitochondrial enzyme succinate dehydrogenase, was assessed using the techniques described by Mosmann [27]. In 96 well microtitre plates, the cell monolayers grew to confluency in 24 hours. Each crude extract was pre-solubilized in Phosphate buffer

saline (PBS) at 37°C, followed by a series of 10-fold dilutions that produced concentrations ranging from 1000-0.01 µg/mL. Cells were then incubated for 72 hours in triplicate with the two extracts and Vincristine (positive control) at different concentrations, and the negative control (growth medium alone) at 37°C in a CO<sub>2</sub> atmosphere. Additionally, the cytopathic effect (CPE), whether it exists or not, was assessed microscopically to determine the viability of the cell. After the treatment period of 72 hours had passed, the supernatants were removed from the wells, and MTT solution (25 µL, 2 mg diluted in 1 mL of PBS) was added to each well. The plates were incubated at 37°C for two hours to dissolve the formazan crystals before DMSO (75 µL) was added to each well. A multi-well spectrophotometer (Multiskan, Thermo Fisher Scientific, Waltham, MA) was used to measure the optical density after the microtitre plates had been shaken for 15 minutes. The 50% cytotoxic concentration (CC<sub>50</sub>) of the extract was found to be the concentration needed to cut the viability of cells in half.

**Statistical Analysis.** All experiments were carried out in triplicates and data were presented as mean ± standard error of the mean (SEM). Data was analysed using GraphPad Prism (6.0) and Dunnett's multiple comparisons of means of each test groups against the control were performed using one-way analysis of variance (ANOVA). A difference was considered to be statistically significant at  $p < 0.05$ .

## **RESULTS**

**Proximate analysis.** The proximate analysis of the samples of green and purple cabbage indicated the percentage of moisture as (17.29% and 14.67%) and ash content of (6.38% and 10.48%), while the proximate analysis of the powdered samples of green and purple cabbage showed that, they contained crude protein (19.27% and 24.07%), crude fibre (13.54% and 15.21%), crude fat (25.08 %

and 17.45%), and carbohydrate (18.43% and 17.66%) (Table 1).

**Elemental analysis.** The elemental analysis result (Table 2) revealed the presence of calcium, magnesium, potassium, sodium, iron, copper, and zinc while lead, cobalt, and chromium were absent in the green and purple cabbage.

**Qualitative phytochemical analysis.** The preliminary phytochemical investigation on

green and purple cabbage extracts revealed the presence of various secondary metabolites such as saponins, flavonoids, tannins, phenolics, terpenoids, steroids, anthraquinones, and quinones. However, alkaloids, coumarins and cardiac glycosides were absent in both extracts while anthocyanins are present in the purple extract but absent in the green extracts (Table 3).

**Table 1:** Proximate Analysis of the Green and Purple Cabbage

Parameters, %	Green Cabbage	Purple Cabbage
Moisture content	17.29±0.03	14.67±0.15
Crude fibre content	13.54±0.07	15.21±0.08
Crude fat	25.08±0.12	17.45±0.07
Ash	6.38±0.16	10.48±0.05
Protein	19.27±0.09	24.07±0.10
Carbohydrate	18.43±0.09	17.66±0.13

**Table 2:** Elemental Analysis of the Green and Purple Cabbage

Parameter	Green Cabbage (mg/g)	Purple Cabbage (mg/g)
Calcium, Ca	5.778	8.551
Magnesium, Mg	2.443	0.874
Potassium, K	21.84	23.12
Sodium, Na	7.516	7.556
Iron, Fe	0.228	0.276
Copper, Cu	0.022	0.014
Zinc, Zn	0.045	0.051

**Table 3:** Qualitative phytochemical analysis of extracts from green and purple cabbages

Parameters	Green Cabbage	Purple Cabbage
Saponin (Froth's Test)	+	+
Alkaloid (Hager's Test)	-	-
Flavonoid (Lead acetate Test)	+	+
Tannin (Braymer's Test)	+	+
Phenolic (Ferric Chloride Test)	+	+
Coumarin (Reaction with 10% NaOH)	-	-
Terpenoid (Salkowaski's test)	+	+
Steroid (Salkowaski's Test)	+	+
Anthraquinone	+	+
Quinone	+	+
Anthocyanin	-	+

+ = Present; - = Absent

**Table 4:** Quantitative phytochemical analysis of green and purple cabbage extracts

Parameter	Green Cabbage (mg/g)	Purple Cabbage (mg/g)
Saponins	4.36±0.04	5.20±0.04
Phenolics	39.9±0.19	55.15±0.20
Tannins	12.29±0.07	15.03±0.14
Flavonoids	12.01±0.12	5.72±0.03

**Table 5:** DPPH radical scavenging effects of green and purple cabbage extracts

Concentration ( $\mu\text{g/mL}$ )	Percentage Inhibition		
	Green Cabbage	Purple Cabbage	Ascorbic Acid
1000	59.01 $\pm$ 0.31	60.11 $\pm$ 0.12	43.10 $\pm$ 0.13
500	53.29 $\pm$ 0.10	55.29 $\pm$ 0.21	37.99 $\pm$ 0.16
250	45.23 $\pm$ 0.28	50.69 $\pm$ 0.18	23.50 $\pm$ 0.11
125	40.86 $\pm$ 0.09	47.45 $\pm$ 0.20	15.78 $\pm$ 0.18
62.5	26.18 $\pm$ 0.17	37.08 $\pm$ 0.28	9.42 $\pm$ 0.30

Values represent the mean of three values of the percentage inhibition at each concentration

**Table 6:** Antioxidant and cytotoxic effects of green and purple cabbage extracts as compared with the standards based on their  $\text{IC}_{50}$  and  $\text{CC}_{50}$  values. n=3

Sample	DPPH, $\text{IC}_{50}$ , $\mu\text{g/mL}$	NO, $\text{IC}_{50}$ , $\mu\text{g/mL}$	RD, $\text{CC}_{50}$ , $\mu\text{g/mL}$
Green cabbage	186.3 <sup>a</sup>	148.7 <sup>a</sup>	10.60 <sup>a</sup>
Purple cabbage	187.6 <sup>a</sup>	103.9 <sup>b</sup>	16.66 <sup>a</sup>
Ascorbic acid	267.0 <sup>b</sup>	135.2 <sup>a</sup>	-
Vincristine	-	-	0.30 <sup>b</sup>

Values carrying different letters within a row are significantly different at  $P > 0.05$ ; DPPH = 2,2-diphenyl-1-picrylhydrazyl; NO = Nitric oxide; RD = Rhabdomyosarcoma;  $\text{IC}_{50}$  = 50% inhibitory concentration;  $\text{CC}_{50}$  = 50% cytotoxic concentration; Ascorbic acid = standard and reference drug for antioxidant agents; Vincristine = standard and reference drug for chemotherapeutic agents

**Phytochemical quantitative analysis.** The saponin, phenolic, and tannin content of purple cabbage was higher than the green cabbage sample as shown in Table 4. However, the flavonoid content in green cabbage was higher than the purple cabbage.

**Antioxidant assays.** The inhibitory concentration at fifty percent ( $\text{IC}_{50}$ ) values of the hydro-methanol extracts on DPPH showed that the green and purple cabbage had a free radical scavenging activity of  $\text{IC}_{50}$  values of 186.3 $\pm$ 0.95  $\mu\text{g/mL}$  and 187.6 $\pm$ 0.99  $\mu\text{g/mL}$  respectively—compared with the standard, ascorbic acid which had an  $\text{IC}_{50}$  of 67  $\pm$  0.88  $\mu\text{g/mL}$ ) as shown in Tables 5 and 6. The extracts of the two varieties of cabbage demonstrated a broad spectrum of nitric oxide scavenging properties (Figure 1 and Table 6). The purple cabbage with  $\text{IC}_{50}$  of 103.9  $\mu\text{g/mL}$ , demonstrated the most significant decrease in the nitric oxide radical compared to the standard, ascorbic acid with  $\text{IC}_{50}$  value of 135.2  $\mu\text{g/mL}$ .

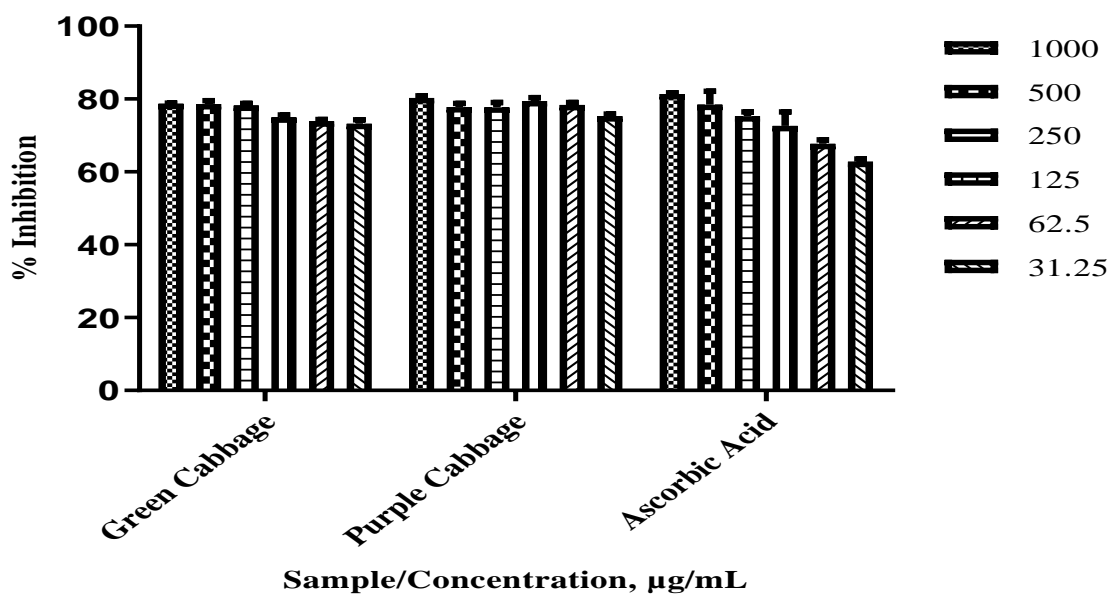
#### Cytotoxicity assay

The result obtained showed that the green purple extract had a  $\text{CC}_{50}$  value of 10.6  $\mu\text{g/mL}$

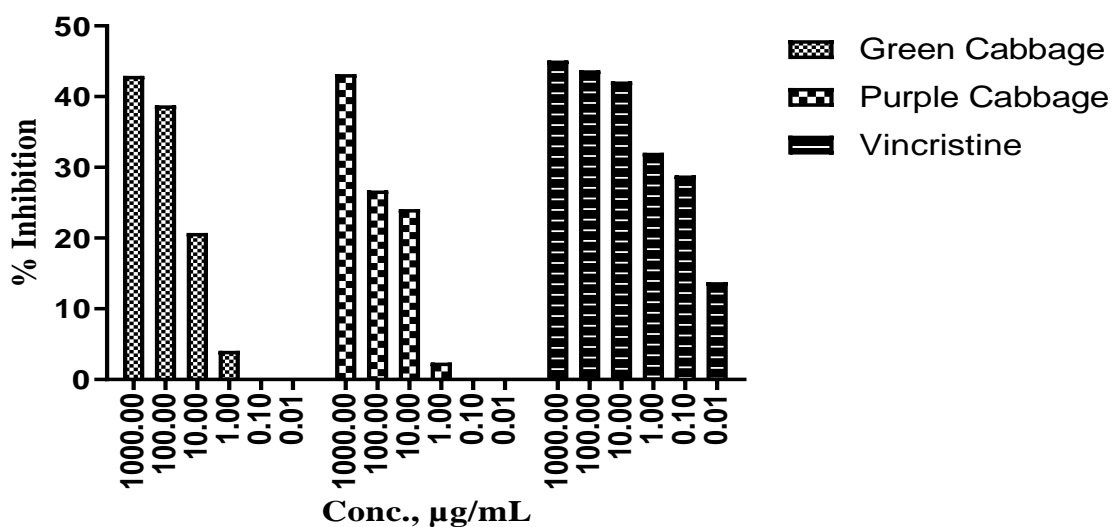
while the purple cabbage had a  $\text{CC}_{50}$  value of 16.66  $\mu\text{g/mL}$  on RD cells as compared to vincristine (standard) with  $\text{IC}_{50}$  of 0.30  $\mu\text{g/mL}$  (Figure 2 and Table 6).

## DISCUSSION

**Proximate analysis.** The high moisture content of the green and purple cabbage allows for enhanced activity of the water-soluble enzymes and co-enzymes required for metabolic activities [28], of the cabbage varieties. It was shown that purple cabbage had more fibre than green cabbage. Crude fibre aids in the prevention of piles, gastrointestinal issues, and constipation. In addition to lowering blood cholesterol and the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer, fibres in the diet are essential for digestion and the efficient removal of wastes [29]. Purple cabbage has a lower crude fat content than green cabbage. Large amounts of (crude) fat consumption is a healthy dietary habit and are advised for people who are overweight or obese [29,30]. Green cabbage has a lower ash percentage than purple cabbage dry matter.



**Figure 1:** Nitric oxide inhibitory effects of green and purple cabbage hydro-methanol extracts as compared with Ascorbic acid (standard), n = 3.



**Figure 2:** Percentage Cytotoxic effects of green and purple cabbage extracts as compared with vincristine (standard) on Rhabdomyosarcoma cells, n=3

The samples are rich in mineral components, according to the ash content range. Similar to this, purple cabbage has a greater crude protein content than green cabbage. The green and purple cabbage's comparatively higher protein content demonstrated their improved nutritional status. Crude protein in leafy vegetables would require dietary

supplementation with proteins from grains and legumes, and plant foods that provide more than 12% of their calorific content from protein have been demonstrated to be good sources of protein [30,31]. One of the most crucial ingredients in many diets is carbohydrate, and digestible carbohydrates are regarded as a significant source of energy which is indicated



in the green and purple cabbage. These figures (green cabbage = 18.43%, purple cabbage = 17.66%) show that they may be a significant source of dietary calories because they are typically regarded as enough.

**Elemental analysis.** Vegetables are important sources of macro and essential trace elements in our diet that are essential for growth, body development, and overall health and well-being [32]. In this study, green and purple cabbage were found to contain significant amounts of these elements. Calcium and magnesium, which are contained in the samples are necessary for proper bone development and structural tissue formation. These two elements also play important roles in the absorption and metabolism of glucose and protein. Deficiencies in calcium and magnesium have been linked to weak bones and connective tissue, hypertension, and poor glucose absorption and utilization [33]. Iron is another essential element found in cabbage that is required for oxygen transportation, proper cellular metabolism, glucose metabolism, and vascular functions. A lack of iron in the body can lead to a weakened immune system, and inhibition of haemoglobin synthesis which can cause anaemia, insomnia, and other health-related complications [34]. Zn and Cu are also essential trace elements that play significant roles in maintaining human health. For example, Zn is an important element in the human body, serving as a cofactor in several enzymatic reactions, including metalloenzymes for carboxyl peptidase, liver alcohol dehydrogenase, and carbonic anhydrase [35]. Copper is also a coenzyme and crucial cofactor in Fe utilization, collagen amalgamation, and concealment of free radicals, and is required for redoxing chemical cytochrome oxidase [35].

Of all the elements quantified, potassium was found to have the highest concentration in both varieties. Potassium is essential in regulating the heartbeat, ensuring

proper function of the muscles and nerves, and is vital for synthesizing protein and metabolism of carbohydrates [36]. Despite the important roles that macro and trace elements play in human health, little is known about the elemental composition and nutritional values of numerous fruits and vegetables in many parts of the world [37]. Most importantly, vegetables may be inadvertently contaminated with chemicals of environmental concern and potentially toxic heavy metals such as lead and Chromium, which are absent in the varieties of cabbage studied.

Vegetables can become contaminated due to environmental pollution, industrial activity, or absorption of heavy metals from polluted soils, industrial waste, or contaminated irrigation water. This has been confirmed by various studies [38,39,40]. Heavy metals have no nutritional value, unlike macro and essential trace elements, and can be found in varying concentrations in a range of food items, such as beverages, juices, wines, and other food products in both developed and developing countries. Heavy metals can also accumulate in human organs and animal tissues through the food chain and trophic levels [41].

**Phytochemical evaluation.** The green and purple cabbage contain secondary metabolites such as phenolics, tannins, and flavonoids, which give them their medicinal value and pharmacological activities ranging from antibacterial and antifungal to antioxidant, anti-inflammatory, antiplatelet, anti-allergic, cytotoxicity, and reduced risk for heart disease [42,43].

The phytochemicals quantified in the two varieties of cabbage, phenolics were the most abundant, while saponins were the least. Saponins are glycosidic and have expectorant and cardiotoxic activity. They have also been reported to have hypoglycaemic and anti-diabetic effects, which can aid in the management of diabetes mellitus. Additionally, a study conducted showed that

saponins was found in beans which may prevent the multiplication of cancer cells by interfering with the replication of cell DNA [44]. Tannins have an antidiarrheal effect and can precipitate proteins in the enterocytes, reducing peristaltic movement and intestinal secretion [45]. Flavonoids, on the other hand, have been found to have potential health benefits like antimicrobial activity, anti-inflammatory, antioxidant, and anti-tumour effects, which are associated with free radical-scavenging action. Flavonoids have also been reported to possess hypoglycaemic and antidiabetic effects [46].

**Antioxidant Assay.** It was observed that the DPPH solution reduced and this is because of the presence of hydrogen donor antioxidant which reacts with free radicals and converts it to non-radical DPPHH form. The DPPH remaining in the reaction mixture measured after some time acts in reverse to the antioxidant radical scavenging activity. The free radical scavenging activity of the extracts from the green and purple cabbage were evaluated using DPPH antioxidant assay. So, these findings from the present study suggested that relatively higher antioxidant potential of the tested samples were involved in the decolorization of the stable DPPH radical solution and these potentials exhibited by the samples could help scavenge free radicals when the body's primary defense mechanisms are overwhelmed by oxidative stress [42]. This study is in accordance with the results of Mihaela et al [47] which shows that selected Brassicaceae vegetables such as white and red cabbages exhibit a relatively high antioxidant capacity. Similarly, the two cabbage extracts in this study exhibited significant nitric oxide inhibitory activity, as compared with the standard drug, ascorbic acid. Nitric oxide performs several functions including the modulation of inflammatory responses. but at an increased level are directly toxic to tissues resulting in vascular damage and other diseases such as cancer. Nitric oxide

is a very unstable species under the aerobic condition. It reacts with  $O_2$  to produce the stable product nitrates and nitrite through the intermediates  $NO_2$ ,  $N_2O_4$  and  $N_3O_4$ . In the presence of a tested agent, which is a scavenger, the amount of nitrous acid decreases. The extent of decrease reflects the extent of scavenging. This study corroborates with the findings on the Tronchuda Cabbage as a scavenger of reactive nitrogen species by Carla et al [48].

**Cytotoxic Assay.** Rhabdomyosarcoma (RD) is a type of sarcoma. Sarcoma is a cancer of soft tissue (such as muscle), connective tissue (such as tendon or cartilage), or bone. RD usually begins in muscles that are attached to bones and that help the body move, but it may begin in many places in the body. About 400 to 500 new cases of RD occur each year in the United States, and more than half of all cases occur in the first decade of life [49,50,51]. RD is a prominent cause of mortality in children in Nigeria and frequently affects the juvenile population [52]. About 3% of all childhood cancers are RD. These tumours are usually embryonal rhabdomyosarcomas (ERD) and tend to develop in the head and neck area or the genital and urinary tracts. Alveolar rhabdomyosarcoma (ARD) affects all age groups and is found more often in the arms, legs, or trunk (chest or abdomen) [49,50]. However, cancer treatments including chemotherapy, immunotherapy, and hormone therapy, have significantly improved patient survival in recent years. These drugs, fluorouracil, capecitabine, carboplatin, doxorubicin, etc. have a significant impact on reducing the mortality of cancer patients, but they have numerous side effects, including nephrotoxicity, hepatotoxicity, cardiotoxicity, etc. Therefore, the challenge in the fight against RD and other cancers is to find anticancer drugs with acceptable toxicity and capable of eliminating sensitive, resistant, and metastatic phenotypic cancers. According to

the CC<sub>50</sub> values, which are less than the American National Cancer Institute's (NCI) standard (CC<sub>50</sub> < 30 µg/mL) for crude extracts [52], the Brassicaceae vegetables used in this study demonstrated significantly higher cytotoxic potentials. Several reports have shown the potential of various Brassicaceae vegetables against different cancer cells [53,54,55], but this study is the first report on the potential of the two cabbage varieties against RD.

**Conclusion.** The results obtained in this study clearly showed that the extracts of green and purple cabbage possessed antioxidant and cytotoxic activity and the results of proximate, elemental, and phytochemical analysis support the use of the two varieties as food supplements.

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