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Article

Comparative phytochemical analysis of some varieties of *Capsicum* species in Akwa Ibom State, Nigeria

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Abstract: Phytochemical and antioxidant analysis of some varieties of *Capsicum* was evaluated. Mature *Capsicum* varieties were collected across the State. The seeds were removed, sun-dried for 3 days, stored for 2 weeks at 15 ºC–25 ºC in polythene bags before planting. Saponins, tannins, flavonoids, alkaloids and cardiac glycosides were present in abundant, moderate and trace amounts. Combined anthraquinones were absent in all varieties. Yellow (0.810 ± 0.0006 µg/mL), red long dry (0.211 ± 0.0006 µg/mL) and round peppers (2.527 ± 0.0003 µg/mL) had the largest values for total phenol, flavonoids and tannins. Shombo and yellow peppers had the largest (0.270 ± 0.002 µg/mL) and least (0.102 ± 0.001 µg/mL) capsaicin content. The antioxidant activities varied across the varieties. At 100 µg/mL of methanol, yellow (45%) and round peppers (45%) had largest mean absorbances for 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity while sub-shombo pepper (23%) had the least. For Ferric Reducing Antioxidant Power (FRAP), yellow (0.63 ± 0.001 µg/mL) and sub-shombo peppers (0.55 ± 0.001µg/mL) had the largest and least values at 100 µg/mL of methanol. At 100 µg/mL of methanol, red long dry (0.112 ± 0.001) and shombo peppers (0.101 ± 0.001) had the largest and least values for the nitric oxide scavenging activity. This study shows that *Capsicum* varieties exhibit bioactive componds similarities and variations with implications in hybridization, taxonomy and conservation.

Keywords: alkaloids; anthraquinones; Capsicum annuum; Capsicum frutescens; capsaicin

1. Introduction

Due to their ability to improve human health and nutrition, plants with a variety of bioactive compounds are becoming more and more popular [1,2]. The first and most important step in any improvement program is to accurately identify the plant species that have medicinal significance, which allows for the reliable and efficient selection of appropriate parental genotypes (with true purity and quality) in plant breeding programs for various nutritional and pharmacological purposes [3]. Many classes of phytochemicals (like phenolic compounds, especially flavonoids and phenolic acid) and antioxidants are found in vegetables and fruits, making them a significant component of the human diet [2]. They are known to protect the body's cells by preventing the oxidation process, which fights against free radicals. Free radicals set off a chain of events that damages cell membranes and disrupts metabolic processes, which increases DNA mutations and changes platelet function, among other impacts [1,2].

As multiple studies have indicated that consuming foods rich in phytochemicals and antioxidants is associated with a reduced risk of certain types of cancer, stroke, and cardiovascular diseases, there is currently significant focus on natural foods,

particularly vegetables rich in these compounds [4]. Numerous studies have detailed the efficacy of the antioxidative components of various pepper species [5]. For example, the inhibitory effect of *C. annuum* var. acuminatum on the enzyme acetylcholinesterase (AChE) was reported by Loizzo et al. [6]. One therapy strategy for managing Alzheimer's disease symptoms is the suppression of this enzyme. The antioxidant properties of *C. annuum* var. acuminatum small and *C. annuum* var. cerasiferum are attributed to their high levels of phenols and flavonoids, which caused the stable free radical DPPH to change into yellow-colored DPPH. Similarly, using the oxygen radical absorbance capacity (ORAC) and DPPH assays, Takahashi et al. [7] reported the excellent antioxidant qualities of C. frutescens fruit extracts from green to red phases.

Peppers contain various bioactive compounds that have the potential to improve health. The distribution of these phytochemicals is determined by genetics and varies depending on the genotype and maturity phase of the plants [8]. Taxonomists use these phytochemicals as markers for classification, and the variation in phytochemical constituents is utilized in taxonomic studies to establish the relatedness between plants. Antioxidants are substances that can either delay or inhibit oxidative damage to a specific molecule. They work by neutralizing free radicals and preventing cell and tissue damage. The body produces its own antioxidants to combat free radicals, while exogenous antioxidants are obtained from food and play a vital role in protecting the body from diseases [9]. Peppers are popular for their abundance of antioxidants, with polyphenols being particularly noteworthy due to their free radical scavenging properties. These compounds also significantly influence the pungency, bitterness, color, and flavor of the fruits, and their levels vary during growth and maturation [10]. *Capsicum*, in particular, contains antioxidants such as tocopherols, ascorbic acid, and b-carotene, which have been found to be effective against cancer, heart disease, and cataracts [11].

Therefore, the goal of the study was to assess the phytochemical and antioxidant properties of various *Capsicum* spp varieties in Akwa Ibom State, Nigeria.

2. Materials and methods

2.1. Study area

Thirteen LGAs in Akwa Ibom State—Uyo, Ikot Ekpene, and Eket—were used to gather the research samples (**Figure 1**). The villages where specimens were collected are listed together with their coordinates in **Table 1**. Akwa Ibom State is situated in southern coastal Nigeria, between latitudes 4°32′ N and 5°33′ N and longitudes 7°25′ E and 8°25′ E. It is roughly 7081 km^2 in size and shares boundaries with Cross River State to the east, Rivers and Abia States to the west, Ebonyi State to the north, and the Bight of Biafra in the Atlantic Ocean to the south. The state receives 3000 mm of rainfall along the coast and 2000 mm inland during the rainy season (April–October) and the dry season (November–March). Temperatures average 25 ℃–28 ℃ year-round, with relative humidity between 75% and 85%.

Figure 1. Map of the study areas [11].

Table 1. Coordinates of the study areas [11].

Local Governments	Latitude (° N)	Longitude (° E)	Altitude (ft)
Uyo Senatorial District			
Uyo	5.015251	7.925669	278
Nsit Ibom	4.869738	7.903226	205
Itu	5.200302	7.977335	86
Ikot Ekpene Senatorial District			
Ikot Ekpene	5.18432	7.716421	355
Ini	5.387142	7.67653	451
Etim Ekpo	4.964826	7.593536	264
Ukanafun	4.87692	7.565049	164
Abak	4.981349	7.781417	219
Eket Senatorial District			
Mbo	4.655009	8.253211	130
Eket	4.657084	7.958585	137
Ibeno	4.544361	7.990957	59
Eastern Obolo	4.522695	7.754939	66
Mkpat Enin	4.65942	7.781625	138

2.2. Collection and propagation of plant materials

(See **Figures 2–10**) 13 Local Government Areas in the three senatorial districts of Akwa Ibom State—Uyo, Ikot Ekpene, and Eket—were used to gather mature fruits from different types of *Capsicum*. Urua Akpan Andem (Uyo), Urua Afaha Offiong (Nsit Ibom), Urua Odot (Nsit Atai), and Urua T-junction Oboetim (Itu) were

the sources of the samples in the Uyo senatorial district. They were gathered from Urua Attor (Ikot Ekpene), Urua Nkari (Ini), Urua Udonkok (Ukanafun), Urua Obo (Etim Ekpo), and Urua Abak (Abak) in the Ikot Ekpene district. Badans Farms (Eket), Urua Enwang (Mbo), Okoroiti (Eastern Obolo), Urua Ukam (Mkpat Enin), and Urua Iwuokpom (Ibeno) were the sources of samples for the Eket district. Before being planted, the seeds were taken out of the fruits, allowed to dry in the sun for three days, and then placed in polythene bags and kept at room temperature (15–25 ºC) for two weeks. From April to September 2020, plants were planted in plastic pots with labels and 3 kg of soil at the greenhouse of the University of Uyo's Department of Botany and Ecological Studies. Harvested mature fruits were subjected to qualitative and quantitative laboratory analysis for phytochemicals and capsaicin [11].

Figure 2. Green small round pepper [11].

Figure 3. Green big round pepper [11].

Figure 4. Yellow pepper [11].

Figure 5. Round pepper [11].

Figure 6. Sub-shombo pepper [11].

Figure 7. Shombo pepper [11].

Figure 8. Red long slender pepper [11].

Figure 9. Red long plumpy pepper [11].

Figure 10. Red long dry pepper [11].

2.3. Preparation of *Capsicum* extracts

The mature fruits of various *Capsicum* varieties were rinsed with distilled water and gently patted dry with a paper towel to eliminate excess moisture. Following this, the fruits were individually air-dried and then crushed using a manual blender. The resulting powder was sealed in labeled plastic bags and stored in the refrigerator

at 4 ºC until extraction using water and ethanol as solvents. Each ground sample weighed 65 g before being put into a separate conical flask with a label and 600 mL of the solvents for extraction. The flasks were shaken on a mechanical shaker for 48 h. Under pressure, the resultant crude extracts were filtered using Whatman No. 1 filter paper, a Buchner funnel and a vacuum pump. A rotary evaporator with a water bath set at 40 ºC was used to concentrate the filtrate to a tenth of its initial volume. A freeze dryer was then used to further dry it. Crude extract, the resultant dried residue, was kept at 4 ºC. For use in the research, aliquot quantities of the leftover crude plant extract were weighed and dissolved in distilled water every day [12].

2.3.1. Qualitative phytochemical screening

To determine the classes of bioactive chemicals present, a phytochemical screening was performed using established procedures on the crude extract of *Capsicum* fruits [13,14].

2.3.2. Test for anthraquinones

Combined anthraquinones: The extract (0.5 g) was heated with 5 mL of aqueous tetraoxosulphate (VI) (10% H_2SO_4) acid and filtered while still hot to combine the anthraquinones. Two millilitres of toluene were used to shake the filtrate. After that, a 10% ammonia solution (1 mL) was added to the toluene layer and shaken. The presence of coupled anthraquinones in the plant extract was indicated by the ammonical layer's pink, red, or violet color [13].

Free anthraquinones: After filtering the 0.5 g of toluene-treated plant extract, 2 mL of a 10% ammonia solution was added to the toluene layer. The presence of free hydroxyl anthraquinones was detected by the ammonical layer turning red, pink, or violet after shaking [13].

2.3.3. Test for saponins

Frothing test: Approximately 0.2 g of the extract was shaken with 5 mL of distilled water in a test tube for 1 min. Frothing which persisted on warming indicated the presence of saponins [13].

2.3.4. Test for tannins

Ferric chloride test: I dissolved around 0.2 g of the extract in 5 mL of distilled water and then filtered it. After that, I added two drops of ferric chloride to the filtrate. I observed a blue-black precipitate, which indicated the presence of tannins [14].

2.3.5. Test for flavonoids

Magnesium metal test: Few pieces of magnesium metal were added to 2 mL of the plant extract solution (prepared by dissolving 0.25 of the extract in 5 mL of distilled water) and then 1 mL of concentrated hydrochloric acid was added. An orange color was obtained indicating the presence of flavonoids [14].

Sodium hydroxide test: Exactly 0.2 g of the extract was dissolved in 2 mL of distilled water and filtered, 1 mL of 5% sodium hydroxide was gently added. A yellow colouration indicates the presence of flavonoids [14].

Ammonia test: Approximately 0.2 g of the extract was dissolved in 3 mL of ethylacetate and warmed. 2 mL of dilute ammonia solution was gently added and the solution shaken. Few drops of 5% potassium hydroxide solution were added

followed by a few drops of dilute hydrochloric acid. A yellow colouration in the lower ammonia layer indicates the presence of flavonoids [14].

2.3.6. Test for cardiac glycosides

Salkowski's test: Approximately 0.2 g of the extract was dissolved in 5 mL of chloroform. 1 mL of concentrated sulphuric acid was gently added by running it down the side of the test tube to form a distinct lower layer. A reddish brown coloration at the interphase indicates the presence of a steroidal ring of the cardiac glycoside [13].

Keller-Killiani test: Approximately 0.2 g of the extract was dissolved in 5 mL of glacial acetic acid containing one drop of ferric chloride solution. The solution was then underlaid with 1 mL of concentrated sulphuric acid by slowly running it down the test tube side to form a distinct lower layer. A brown ring at the interphase indicates the presence of a deoxy sugar characteristic of the cardiac glycoside [14].

Lieberman's test: 5 mL of acetic anhydride were used to dissolve around 0.2 g of the extract, which was then thoroughly chilled in ice. To create the lower layer, precisely 1 mL of concentrated tetraoxosulphate (VI) acid was added. The existence of the aglycone component of the cardiac glycoside, the steroidal nucleus, was demonstrated by a color shift from violet to blue to green [14].

2.3.7. Test for alkaloids

In a test tube set over a boiling water bath, around 0.2 g of the extract was heated with 5 mL of 5% HCl. After letting the mixture cool, it was filtered. Dragendorff's precipitating reagent was added in little drops to the filtrate, and the results were monitored. Alkaloids were thought to be present when a red or orange precipitate formed [14].

2.4. Quantitative phytochemical screening

2.4.1. Determination of total phenolic content

Using spectrophotometry and the Folin-Ciocalteau reagent, the total phenolic content of the seed's crude extract and fractions was determined. This was accomplished by combining 2.5 mL of 10% Folin-Ciocalteau, 2 mL of 7% Na_2CO_3, and 0.5 mL (1 mg/mL) of the crude extract and fractions. After 15 s of vortexing, the resultant liquid was incubated for 30 min at 40 ºC to develop its color. A wavelength of 765 nm was then used to measure the samples' absorbance. 2.5 mL of Folin-Ciocalteau reagent was added at various quantities (20–100 µg/mL) for the gallic acid calibration curve. The calibration curve was then used to determine the total phenolic content, and the results were reported as milligrammes of gallic acid equivalent per gramme of dry weight. Each of these processes was performed three times [4].

2.4.2. Determination of total tannins

The analysis was carried out following the Van–burden and Robinson [15] method. A 5 g sample was placed into a 50 mL conical flask. A mechanical shaker was used to stir the liquid for 1 h after precisely 50 mL of distilled water were added. The solution was then adjusted to the proper level after being filtered into a 50 mL volumetric flask. A test tube was then filled with 5 mL of the filtered solution, 2 mL

of 0.1 M $FeCl_3$ in 0.1 M HCl, and 0.008 M potassium ferrocyanide. After 10 min, the absorbance at 395 nm was measured.

2.4.3. Determination of total flavonoids

The method outlined by Zhishen et al. [16] was employed to estimate the flavonoid content. 150 µL of a 5% sodium nitrite solution was added after 200 µL of distilled water and 1 mL of plant extracts were combined separately. 150 µL of a 10% aluminium chloride solution was added after a 5-min incubation, and then the mixture was let to stand for 6 min. After that, 2 mL of a 4% sodium hydroxide solution was added, and distilled water was added to bring the solution up to 5 mL. Following a good shake, the mixture was allowed to remain at room temperature for 15 min before the flavonoid absorbance at 510 nm was determined. The appearance of a pink color suggested the presence of flavonoids. A standard curve was used to calculate the extract's rutin equivalent/mg (RE/g) based on dry weight, which represents the overall flavonoid content.

2.4.4. Statistical data analyses

With Graphpad Prism (6.0), means, standard errors, and two-way analysis of variance (ANOVA) were performed.

3. Results

3.1. Phytochemical constituents of the *Capsicum* varieties

Qualitative phytochemical constituents of the Capsicum varieties: The qualitative phytochemical constituents are presented in **Tables 2–12**.

Test for saponins: In testing the presence of saponin in the *Capsicum* varieties, green small round pepper, green big round pepper, shombo pepper, red long slender pepper, red long plumpy pepper and red long dry pepper had abundance of saponin while yellow, round and sub-shombo peppers had moderate amount of saponin (**Table 2**).

Table 2. Composition of saponins in the *Capsicum* varieties.

Varieties	Inference
Green small round pepper	+++
Green big round pepper	+++
Yellow pepper	++
Round pepper	++
Sub-shombo pepper	++
Shombo pepper	+++
Red long slender pepper	+++
Red long plumpy pepper	+++
Red long dry pepper	+++

Note: Abundant (+++) Moderate (++).

Test for tannins: **Table 3** displays the results of the tannin test conducted on the various kinds of *Capsicum*. Round and shombo peppers showed trace quantities of tannin, whereas green small round, green big round, yellow, and red long dry

peppers had a lot of tannin. Sub-shombo peppers, red long slender peppers, and red long dry peppers had moderate amounts of tannin.

Table 3. Composition of tannins in the *Capsicum* varieties.

Varieties	Inference
Green small round pepper	+++
Green big round pepper	+++
Yellow pepper	+++
Round pepper	+
Sub-shombo pepper	++
Shombo pepper	+
Red long slender pepper	++
Red long plumpy pepper	++
Red long dry pepper	+++

Note: Abundant (+++), Moderate (++) Trace (+).

Test for flavonoids using magnesium metal test: The test for flavonoids using magnesium metal test is presented in **Table 4**. From the result, flavonoids were moderate in green small round pepper, green big round, sub-shombo and shombo peppers had trace amount of flavonoids while yellow, round, red long slender, red long plumpy and red long dry peppers had no flavonoids in them.

Table 4. Composition of flavonoids in the *Capsicum* varieties using magnesium metal test.

Varieties	Inference
Green small round pepper	++
Green big round pepper	+
Yellow pepper	-
Round pepper	-
Sub-shombo pepper	+
Shombo pepper	+
Red long slender pepper	-
Red long plumpy pepper	-
Red long dry pepper	-

Note: Moderate (++), Trace (+), Absent (-).

Test for flavonoids using sodium hydroxide test: The test for the presence of flavonoids in the *Capsicum* varieties using sodium hydroxide test is presented in **Table 5**. The result showed that flavonoids were present in moderate amounts in green small round, green big round pepper, yellow pepper, round pepper, sub-shombo, red long slender pepper, red long plumpy and red long dry peppers while shombo pepper had trace amount of flavonoids.

Table 5. Composition of flavonoids in the *Capsicum* varieties using sodium hydroxide test.

Varieties	Inference
Green small round pepper	++
Green big round pepper	++
Yellow pepper	++
Round pepper	++
Sub-shombo pepper	++
Shombo pepper	+
Red long slender pepper	++
Red long plumpy pepper	++
Red long dry pepper	++

Note: Moderate (++) Trace (+).

Test for flavonoids using ammonia: The result for the test of flavonoids using ammonia is presented in **Table 6**. Flavonoids were present in trace amounts in green small round and yellow peppers but absent in green big round, round, sub-shombo, shombo, red long slender, red long plumpy and red long dry peppers.

Table 6. Composition of flavonoids in the *Capsicum* varieties using ammonia test.

Varieties	Inference
Green small round pepper	+
Green big round pepper	-
Yellow pepper	+
Round pepper	-
Sub-shombo pepper	-
Shombo pepper	-
Red long slender pepper	-
Red long plumpy pepper	-
Red long dry pepper	-

Note: Trace (+), Absent (-).

Test for alkaloids: The test for alkaloids in the *Capsicum* Red long dry peppers presented in **Table 7**. From the result, alkaloids were abundant in yellow pepper, round pepper and red long plumpy pepper, moderate in green small round pepper, sub-shombo pepper, shombo pepper, red long slender pepper and red long dry pepper while green big round pepper had alkaloids in trace amounts.

Table 7. Composition of alkaloids in the *Capsicum* varieties.

Varieties	Inference
Green small round pepper	++
Green big round pepper	+
Yellow pepper	+++
Round pepper	+++
Sub-shombo pepper	++
Shombo pepper	++
Red long slender pepper	++
Red long plumpy pepper	+++
Red long dry pepper	++

Note: Abundant (+++), Moderate (++) Trace (+).

Test for cardiac glycosides using Salkowski's test: The test for cardiac glycosides using Salkowski's test is presented in **Table 8**. Cardiac glycosides were abundant in green small round pepper, green big round pepper, yellow pepper and round pepper, moderate in shombo pepper and red long dry peppers while sub-shombo, red long slender and red long plumpy peppers had trace amounts of cardiac glycosides.

Table 8. Composition of cardiac glycosides in the *Capsicum* varieties using Salkowski's test.

Varieties	Inference
Green small round pepper	+++
Green big round pepper	+++
Yellow pepper	+++
Round pepper	+++
Sub-shombo pepper	+
Shombo pepper	++
Red long slender pepper	+
Red long plumpy pepper	+
Red long dry pepper	++

Note: Abundant (+++), Moderate (++), Trace (+).

Test for cardiac glycosides using Keller-Killiani test: The test for cardiac glycosides in the *Capsicum* varieties using Keller-Killiani test is presented in **Table 9**. Cardiac glycosides were abundant in all the varieties.

Table 9. Composition of cardiac glycosides in the *Capsicum* varieties using Keller-Killiani test.

Varieties	Inference
Green small round pepper	+++
Green big round pepper	+++
Yellow pepper	+++
Round pepper	+++
Sub-shombo pepper	+++
Shombo pepper	+++
Red long slender pepper	+++
Red long plumpy pepper	+++
Red long dry pepper	+++

Note: Abundant (+++).

Test for cardiac glycosides using Lieberman's test: The result for the presence of cardiac glycosides in the *Capsicum* varieties using Lieberman's test is presented in **Table 10**. Cardiac glycosides were abundant in red long dry pepper, moderate in green big round, yellow, round, sub-shombo, shombo, red long slender pepper and red long plumpy peppers while green small round pepper had trace amounts of cardiac glycosides

Table 10. Composition of cardiac glycosides in the *Capsicum* varieties using Lieberman's test.

Varieties	Inference
Green small round pepper	+
Green big round pepper	++
Yellow pepper	++
Round pepper	++
Sub-shombo pepper	++
Shombo pepper	++
Red long slender pepper	++
Red long plumpy pepper	++
Red long dry pepper	+++

Note: Abundant (+++), Moderate (++), Trace (+).

Test for combined anthraquinones: The result for the test of combined anthraquinones in the red long dry peppers presented in **Table 11**. Combined anthraquinones were absent in all the species

Table 11. Composition of Combined anthraquinones in the *Capsicum* varieties.

Varieties	Inference
Green small round pepper	Absent
Green big round pepper	Absent
Yellow pepper	Absent
Round pepper	Absent
Sub-shombo pepper	Absent
Shombo pepper	Absent
Red long slender pepper	Absent
Red long plumpy pepper	Absent
Red long dry pepper	Absent

Test for free anthraquinones: The result for the test of free anthraquinones in the *Capsicum* varieties are presented in **Table 12**. Free anthraquinones were absent in all the species.

Table 12. Composition of free anthraquinones in the *Capsicum* varieties.

Varieties	Inference
Green small round pepper	Absent
Green big round pepper	Absent
Yellow pepper	Absent
Round pepper	Absent
Sub-shombo pepper	Absent
Shombo pepper	Absent
Red long slender pepper	Absent
Red long plumpy pepper	Absent
Red long dry pepper	Absent

Quantitative phytochemical constituents of the Capsicum varieties: The quantitative physicochemical constituents of the *Capsicum* sp. is presented in **Table 13**. The phytochemical contents analyzed for, were total phenolic, total flavonoids and total tannins. The total phenolic contents of the *Capsicum* varieties ranged from 0.599 ± 0.0006 µg/mL to 0.810 ± 0.0006 µg/mL. Yellow pepper and shombo pepper had the highest (0.810 ± 0.0006 µg/mL) and least (0.599 ± 0.0006 µg/mL) values for total phenolic content. The total phenolic contents across the species were significantly different ($p < 0.05$).

Table 13. Phytochemical contents of nine *Capsicum* varieties.

Varieties	Total phenol (µg/mL)	Total flavonoids (µg/mL)	Total tannins (µg/mL)
Green small round pepper	0.655 ± 0.0009^{a}	0.061 ± 0.0003^{a}	1.473 ± 0.0003^{a}
Green big round pepper	0.618 ± 0.0009^{b}	0.059 ± 0.0003^{a}	1.595 ± 0.0009^{b}
Yellow pepper	0.810 ± 0.0006^{c}	0.065 ± 0.0006^{b}	1.514 ± 0.0009^{c}
Round pepper	0.711 ± 0.0006^{d}	0.062 ± 0.0003^{a}	2.527 ± 0.0003^{d}
Sub-shombo pepper	0.623 ± 0.0006^{e}	0.048 ± 0.0006^{c}	1.519 ± 0.0006^{e}
Shombo pepper	0.599 ± 0.0006^{f}	0.041 ± 0.0003^{d}	1.567 ± 0.001^{f}
Red long slender pepper	0.695 ± 0.0006^{g}	0.064 ± 0.0006^{b}	1.524 ± 0.0006^{g}
Red long plumpy pepper	0.652 ± 0.0006^{h}	0.065 ± 0.0003^{b}	1.553 ± 0.001^{h}
Red long dry pepper	0.747 ± 0.0006^{i}	0.211 ± 0.0006^{e}	1.669 ± 0.002^{i}

Note: Means with different superscripts along the same column are significantly different ($p < 0.05$) ± Standard error.

The total flavonoids in the species ranged between 0.041 ± 0.0003µg/mL and 0.211 ± 0.0006 µg/mL. Red long dry pepper had the highest total flavonoids of 0.211 ± 0.0006 µg/mL while shombo pepper had the least total phenolic content (0.041 ± 0.0003 µg/mL). The total phenolic contents across the species were significantly different ($p < 0.05$).

For the total tannins, the values across the *Capsicum* varieties ranged between 1.473 ± 0.0003 µg/mL and 2.527 ± 0.0003 µg/mL. Round pepper and green small round pepper had the highest (2.527 ± 0.0003 µg/mL) and least (1.473 ± 0.0003 µg/mL) values, respectively. However, the values obtained for total tannins across the species were significantly different ($p < 0.05$). The phytochemical assay in the *Capsicum* varieties also revealed variations in the presence of certain phytochemicals both quantitatively and qualitatively. Qualitatively, phytochemicals such as saponins, tannins, flavonoids, alkaloids and cardiac glycosides were present in abundant, moderate and trace quantity in some species. This confirms the presence of secondary metabolites in the pepper varieties in varying amounts. Since these secondary metabolites are reported to have many biological and therapeutic properties [17,18], this may go a long way to confirm the medicinal properties of these pepper varieties [19]. The flavonoids serve as powerful antioxidants that combat free radicals and prevent oxidative damage to cells, exhibiting potent anti-cancer properties [20]. Alkaloids are effective therapeutic compounds with strong antibacterial characteristics, making them essential medicinal agents when isolated in their pure form [21]. Due to their impact on the heart, glycosides hold significant medicinal value, while tannins play a crucial role in inhibiting bacteria from attaching to the urinary tract walls, thus preventing urinary tract infections [19]. The abundance of saponin in *Capsicum* varieties (green small round pepper, green big round pepper, shombo pepper, red long slender pepper, red long plumpy pepper) confirms their important dietary properties, as its presence in food helps in reducing cholesterol levels which is beneficial to health [22].

3.2. Capsaicin content in the *Capsicum* varieties

The capsaicin content in the studied *Capsicum* varieties is presented in **Table 14**. The values ranged from 0.102 ± 0.001 µg/mL to 0.270 ± 0.002 µg/mL. Shombo pepper and yellow pepper had the highest (0.270 ± 0.002 µg/mL) and least (0.102 ± 0.001 µg/mL) capsaicin contents, respectively.

Table 14. Capsaicin content of the *Capsicum* varieties.

Varieties	Concentration (µg/mL)
Green small round pepper	0.132 ± 0.001
Green big round pepper	0.129 ± 0.002
Yellow pepper	0.270 ± 0.002
Round pepper	0.116 ± 0.001
Sub-shombo pepper	0.125 ± 0.002
Shombo pepper	0.102 ± 0.001
Red long slender pepper	0.122 ± 0.001
Red long plumpy pepper	0.144 ± 0.001
Red long dry pepper	0.109 ± 0.001

The levels of total phenols, total flavonoids, and total tannins exhibited significant variation among the pepper varieties studied ($p < 0.05$). Researchers have taken a keen interest in phenols, which are a key phytochemical component found in various plants, due to their strong antioxidant properties that are associated with preventing specific diseases in the human body, including cancer [23,24]. Yellow pepper had the highest total phenol content, yellow and red long plumpy peppers had the highest total flavonoids while round pepper had the highest total tannin content. This variability in pepper varieties for phenols had been alluded by Zhuang et al. [23].

The presence of total phenols confirms that peppers are important source of this secondary metabolite, which are mainly localized in the peels [25]. The concentration of total phenols was highest in yellow pepper. This goes a long way to confirm that total phenols in pepper depends upon the varieties and color of the fruit [26]. The high concentration of total phenols in the yellow pepper in this study, contrasts with the findings of Blanco-Ríos et al. [27] and Zaki et al. [26]. While the former reported a higher total phenols in bell peppers with red color, followed by yellow, and then by green, the latter reported a higher total phenols in Orion (green pepper). The variations in total phenols in the pepper varieties may be attributed to inherent genetic differences in the cultivars, agronomic conditions, maturity, post-harvest handling and pre and post-harvest treatments applied to the fruit [23]

60% of the total phenolics are contributed by flavonoids, making them the most abundant naturally occurring phenolics. Their ability to scavenge free radicals is due to their biological functions, which encompass antioxidant, anticancer, and anti-inflammatory properties [28]. The total flavonoid varied across the studied pepper varieties. Red long dry pepper had the highest concentrations of flavonoids while shombo pepper had the least concentration of flavonoid. These variations in flavonoid content are also related to maturity period of the pepper, type of cultivar

and growing conditions [29]. For total tannin content, variations were also observed across the varieties with round pepper and green small round pepper having the highest and least concentrations. These variations among *Capsicum* varieties with regards to tannin content, may expound their different cultivars, growth conditions and maturity stages. On the whole, the closeness in values of *Capsicum* varieties with regards to these compounds (total phenols, flavonoids and tannins) may depict their genetic relatedness while those with wide variations may portray their genetic dissimilarities. This agrees with the findings of Scalzo et al. [30].

Overall, this research highlights the potential of *Capsicum* as a valuable source of bioactive compounds with significant health benefits. It is recommended that; Incorporating a variety of *Capsicum* colors (red, yellow, green, orange) into meals to benefit from a wider range of phytochemicals, use minimal processing techniques (e.g., blanching, freezing) to preserve nutrients and flavor, collaborate with food scientists and nutritionists to optimize product formulation and labeling. By applying this knowledge, we can improve agricultural practices, develop superior cultivars, and promote healthier diets. Thus, future research can contribute to the development of improved *Capsicum* varieties with enhanced nutritional and health benefits, as well as sustainable agricultural practices.

4. Conclusions

Assessing the variability of plant bioactive compounds has been effectively employed as an extra method to categorize certain plant species. This research demonstrated the value of examining phytochemical content, antioxidant properties, and capsaicin levels in the ongoing effort to thoroughly characterize and classify different types and species of *Capsicum*. Conclusively, this study shows that these *Capsicum* varieties exhibit a considerable amount of similarities and variations based on their phytochemical, antioxidant and capsaicin properties.

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References

1. Idris OA, Wintola OA, Afolayan AJ. Phytochemical and antioxidant activities of Rumex crispus L. in treatment of gastrointestinal helminths in Eastern Cape Province, South Africa. Asian Pacific Journal of Tropical Biomedicine. 2007; 7(12): 1071–1078.
2. Unuofin JO, Otunola GA, Afolayan AJ. Phytochemical screening and in vitro evaluation of antioxidant and antimicrobial activities of Kedrostis africana (L.) Cogn. Asian Pacific Journal of Tropical Biomedicine. 2017; 7(10): 901–908.
3. Ganiea SH, Upadhyaya P, Dasa S, Sharmab MP. Authentication of medicinal plants by DNA markers. Plant Gene. 2015; 4: 83–99.
4. Kaur C, and Kapoor HC. Antioxidants in fruits and vegetables—the millennium's health. International Journal of Food Science and Technology. 2001; 36(7): 703–725.

5. Khan FA, Mahmood T, Ali M, et al. Pharmacological importance of an ethnobotanical plant: Capsicum annuum L. Natural Product Research. 2014; 28(16): 1267–1274.
6. Loizzo MR, Tundis R, Menichini F, et al. Influence of ripening stage on health benefits properties of Capsicum annuum var. cuminatum L.: In vitro studies. Journal of Medicinal Food. 2008; 11(1): 184–189.
7. Takahashi M, Arakaki M, Yonamine K, et al. Influence of fruit ripening on colour, organic acid contents, capsaicinoids, aroma compounds, and antioxidant capacity of shimatogarashi (Capsicum frutescens). Journal of Oleo Science. 2018; 67(1): 113–123.
8. Patthamakanokporn O, Puwastien P, Nitithamyong A, and Sirichakwal PP. Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. Journal of Food Composition and Analysis. 2008; 21(3): 241–248.
9. Arshiya S. The Antioxidant Effect of Certain Fruits. Journal Pharmaceutical Science and Research. 2013; 5(12): 265–268.
10. Estrada B, Pomar F, Díaz J, et al. Pungency level in fruits of the Padrón pepper with different water supply. Scientia Horticulturae. 2000; 81(4): 385–396.
11. Bright FA, Kingsley A, Okon OG. Growth Analysis and Characterization of Some Varieties of Capsicum Species in Akwa Ibom State, Nigeria. Asian Journal of Biological Sciences. 2024; 17(4): 796–808.
12. Maforimbo E. Evaluation of Capsicum as a source of natural antioxidant in preventing rancidity in sunflower oil. Journal of Food Technology in Africa. 2002; 7(2): 68–72.
13. Sofowora LA. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd; 1993. pp. 55–71.
14. Trease GE, and Evans WO. Trease and Evans Pharmaacognosy, 16th ed. Sauders Elsevier Limited; 2009. pp. 104–262.
15. Van-Burden TP, and Robinson WC. Formation of complexes between proteins and tannic acid. Journal of Agricultural and Food Chemistry. 1981; 1: 77–81.
16. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64(4): 555–559.
17. Benedec D, Vlase L, Oniga I, et al. Polyphenolic composition, antioxidant and antibacterial activities for two Romanian subspecies of Achillea distans Waldst. et Kit. ex Wild. Molecules. 2013; 18(8): 8725–8739.
18. Vishnu R, Nisha R, Jamuna S, Paulsamy S. Quantification of total phenolics and flavonoids and evaluation of in vitro antioxidant properties of methanolic leaf extract of Tarenna asiatica-an endemic medicinal plant species of Maruthamali hills, Western Ghats, Tami Nadu. Journal of Research in Plant Sciences. 2013; 2(2): 196–204.
19. Emmanuel-Ikpeme C, Henry P, Okiri OA. Comparative evaluation of the nutritional, phytochemical and microbiological quality of three pepper varieties. Journal of Food and Nutrition Sciences. 2014; 2(3): 74–80.
20. Salah N, Miller NJ, Pagange G, et al. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidants. Archives of Biochemistry and Biophysics. 1995; 322(2): 339–346.
21. Stray F. The natural guide to medicinal herbs and plants. Tiger Book International; 1998. pp. 12–16.
22. Milgate J, Roberts D. The nutritional and biological significance of saponins. Nutritional Research. 1995; 15: 1233–1249.
23. Zhuang Y, Chen L, Sun L, Cao J. Bioactive characteristics and antioxidant activities of nine peppers. Journal of Functional Foods. 2012; 4(1): 331–338.
24. Ohikhena F, Wintola O, Afolayan AJ. Quantitative phytochemical constituents and antioxidant activities of the mistletoe, Phragmanthera capitata (sprengel) balle extracted with different solvents. Pharmacognosy Research. 2018; 10(1): 16–23.
25. Marín A, Ferreres F. Tomás-Barberán FA, Gil MI. Characterization and quantitation of antioxidant constituents of sweet pepper (Capsicum annuum L.). Journal of Agricultural and Food Chemistry. 2004; 52(12): 3861–3869.
26. Zaki N, Hasib A, Eddine KC, et al. Comparative evaluation of the phytochemical constituents and the antioxidant activities of five Moroccan Pepper Varieties (Capsicum annuum L.). Journal of Chemical, Biological and Physical Sciences. 2017; 7(4): 1294–1306.
27. Blanco-Ríos AK, Medina-Juarez LA, González-Aguilar GA, Gamez-Meza N. Antioxidant activity of the phenolic and oily fractions of different sweet bell peppers. Journal of Mexican Chemical Society. 2013; 57: 137–143.
28. Righetto AM, Netto FM, Carraro F. Chemical composition and antioxidant activity of juices from mature and immature Acerola (Malpighia emarginata DC). Food Science and Technology International. 2005; 11: 315–321.
29. Scalzo J, Politi A, Pellegrini N, et al. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition Journal. 2005; 21(2): 207–213.
30. Sharma K, Assefa AD, Kim S, et al. Evaluation of total phenolics, flavonoids and antioxidant activity of 18 Korean onion cultivars: A comparative study. Journal of the Science of Food and Agriculture. 2014; 94(8): 1521–1529.