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Phytochemical and Antioxidant Evaluation of Varieties of Pepper Fruits in Akpan Andem Market in Uyo Akwa Ibom State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OTU and APE designed the study. Author OTU wrote the protocol and wrote the first draft of the manuscript. Authors VEU, EEE, AOO and EPOO managed chemical analyses of the study. Authors VEU and SAI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The results of this research showed significant difference in the phytochemical contents of *Capsicum baccatum* L. (Yellow), *Capsicum baccatum* L. (Red), *Capsicum Chinese* Jacq., *Capsicum annuum* L. (Cayenne), *Capsicum annuum* L. (Bell) in terms of total Saponin, Tanins and Cardiac glucosides. The concentrations of flavonoids, Alkaloids and Anthraquinones were similar in the five species of *Capsicum* studied and it could be attribute to cultivation, ripeness, storage and soil salinity, among other factors. Reducing power assay is one of established method for evaluation of antioxidant potential of a test sample which was employed in the course of this work. Basically, it involves reduction of Fe^{3+} into Fe^{2+} with the formation of Perl's Prussian blue colour complex wherein absorbance is read at 700 nm. This reducing ability varies with respect to various concentrations of antioxidant present in the samples. The Different fruit composition of the five pepper species indicates that apart from the evident morphological differences in terms of fruit shape and appearance, they also differ in their content of phytochemicals.

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Keywords: Akpan Andem Market; Akwa Ibom State; capsicum; phytochemical; DPPH scavenging.

1. INTRODUCTION

Currently, people have interest in maintaining good health and an excellent body figure, therefore, they have become more careful in the food they choose to consume, looking for food with a high nutritional value, bioactive compounds and antioxidant capacity, such as fruits and vegetables. Epidemiological studies have consistently demonstrated a positive relation between the consumption of fruits and vegetables and a reduction in the mortality rate due to heart disease, cancer, and other degenerative diseases, as well as aging [1]. This is attributed to the fact that these foods are the main source of nutraceutical compounds, such as vitamins, minerals, and phenolic compounds, natural antioxidants, fiber, and other biotic compounds [1,2].

Peppers (hot and sweet) belong to the Solanaceae family, genus *Capsicum* [3]. This genus originated from Central and South America [4] and comprises about 30 species, of which, five domesticated that comprise *C. annuum* L. (hot and sweet peppers), *Capsicum frutescens* L. or bird pepper, *C. chinense* Jacq. or aromatic chili pepper, *Capsicum baccatum* L. (aji), and *C. pubescens* Ruiz and Pav. (rocoto). The first three species are the most cultivated in both tropical and temperate zones. *C. annuum* often forms a complex with *C. frutescens* and *C. chinense*. In Africa, they are generally considered together as *C. annuum* L [4].

The crop spread rapidly across Europe into India, China, and Japan. The new spice, unlike most of the *Solanums* from the Western Hemisphere, was incorporated into the cuisines instantaneously. Probably for the first time, pepper was no longer a luxury spice only the rich could afford. Since its discovery by Columbus, the crop has been incorporated into most of the world's cuisines. It has been commercially grown in the United States, when Spanish colonists planted seeds and grew Chile using irrigation from the Rio Chama in northern New Mexico [5].

The bell pepper (*C. annuum* L) is a fruit well known for its high content in bioactive compounds and strong antioxidant capacity and it is among the most popular of fresh vegetables worldwide due to its combination of color, flavor, and nutritional value [6]. The plant, native to North and South America, is most productive in warm, dry climates and is used both medicinally as well as a food in Africa and other countries of the world [7]. Currently, a broad number of varieties are available in the supermarkets, most of which change from a green color to yellow, orange, red, or purple when they are completely ripe. Green peppers are often harvested before they complete ripening, and the maturity stage can partly account for the content in phytonutrients and thus the consumption of antioxidants in the diet [8].

Fresh peppers have exceptionally high quantities of ascorbic acid and their attractive red color is due to several carotenoid pigments that include β-carotene with pro-vitamin A activity and oxygenated carotenoids such as capsantine, capsorubin, and cryptocapsin, which are exclusive to these fruits and have proven to be

Fig. 1. *Capsicum baccatum* L. (Red)

Fig. 2. *Capsicum baccatum* L. (Yellow)

Fig. 3. *Capsicum annuum* L. (Bell)

Fig. 4. *Capsicum annuum* L. (Cayenne)

Fig. 5. *Capsicum Chinese* Jacq

effective at scavenging free radicals [9]. Peppers also contain large quantities of neutral phenolic compounds or flavonoids called quercetin, luteolin, and capsaicinoid [10]. The consumption of these bioactive compounds provide beneficial effects in human health due to their antioxidant properties, which protect against the oxidative damage to cells and thus prevent the development of common degenerative diseases such as cancer, cardiovascular diseases, cataracts, diabetes, Alzheimer's, and Parkinson's [6]. These chemical compounds also prevent the oxidation of essential fats within the cells of the brain that are considered necessary for its optimal functioning [11].

Peppers (*C. annuum*) present an important genetic variability of wild and cultivated accessions that differ in their vegetative growth (determined, sympodial, fascicular, etc.), their criteria of fruit quality (shape, weight, length, color, taste, etc.) and their marketable output [12, 13]. This variability is not largely appreciated in pepper improvement programs [14].

The aim of this Study is to carry out phytochemical and antioxidant evaluation of varieties of fruits of pepper in Akpan Andem market in Uyo, Akwa Ibom State.

2. MATERIALS AND METHODS

2.1 Materials

Solvent jar, glass funnels, conical flask, test tubes, test tube racks, filter paper, (Whatman I Springhfield Mill, England) Digital water bath (Thomas Scientific United Kingdom), Aluminum foil, cotton wool, masking tape, spatula, sample bottles, syringes and needles, blender, drying

oven (Memmert UN 55), beaker, digital Weighing balance (ESJ210-4A) Ultraviolet spectrometer (Unico UV-2100 Spectrophotometer, shangai instrument co. ltd., china) capillary tubes, dropper, glass rod.

2.2 Chemical and Reagent

Acetic anhydride, ferric chloride, toluene, glacial acetic acid, acetone, hydrogen tetraoxosulphate vi acid, sodium hydroxide, hydrochloric acid, ammonia (novora group ltd., England) Butanol, N- hexane, methanol, ethanol, chloroform, ethylacetate, (IHD,India), 2,2- diphynyl-1-picrythydrazyl (DPPH) (Sigma, Aldrich Inc., Germany), Magnesuim metal (Phil Haries ltd., England). Dragendorff"s reagent, distilled water, Ascorbic acid Consentrated Sulphuric acid, mayers reagent.

2.3 Plant Collection

The fruits of different species of pepper used for this work, were bought from traders in Akpan Andem Market, Uyo local government area, Akwa ibom state, Nigeria.

2.4 Extraction

Maceration process of extraction, was carried out. The samples were blended into a not very smooth paste. It was then put into a solvent jar. The solvent for extraction was added which is this case is Methanol. The mixture was then allowed for 72 hours, with intermittent shaking. It was then filtered to obtain filtrate. The filtrate was then concentrated to dryness to obtain the extract.

2.5 Phytochemical Screening

The phytochemical screening was carried out on the fruit extracts of the 5 species of *Capsicum* according to standard methods to identify the classes of bioactive compounds present [15,16].

2.6 Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The Ferric reducing antioxidant power activity of the plant was determined using the method of [17]. Various concentrations (20,40,60,80,100 µg/ml) of pepper species, *C. baccatum* (yellow), *C. baccatum* (red), *C. Chinese*, *C. annuum* (cayenne) and *C. annuum* (bell), fractions, extract and ascorbic acid (2.5 ml) we're mixed individually with the mixture containing 2.5 ml of 0.2ml of sodium phosphate buffer (pH 6.6) and 2.5ml of potassium ferricyanide ($k_3$fe(CN)6) 1%w/v). The resulting mixture was incubated at 50c for 20 minutes, followed by the addition of 2.5 ml of trichloreactic acid (10%w/v). Which was then centrifuged at 650 rpm for 10minutes. The upper layer of the lotion, was mixed with 2.5 ml of distilled water and 0.5 ml of Ferric chloride (0.1%wv) the absorbance was then measured at the wavelength of 700nm against a blank sample. The Assays were carried out in triplicate.

2.7 Determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH free radical scavenging effect of the species *C. baccatum* (yellow), *C. baccatum* (red), *C. Chinese*, *C. annuum* (cayenne) and *C. annuum* (bell) fractions, extracts and ascorbic acid prepared in methanol at various concentration (20,40,60,80,100 µg/ml) were evaluated according to the method of Shekhar and Anju [18]. 2,2-Dipheyl-l-picrylhydrazyl (0.1 mm, 1 ML) was added to 3 ml of the solution prepared with *C. baccatum* (yellow), *Capsicum baccatum* (red), *C. Chinese*, *C. annuum* (cayenne) and *C. annuum* (bell) fractions extract and ascorbic acid stirred for 1 minute each mixture was incubated in the dark for 30 minutes and the absorbance was measured at 517 nm. The assays were carried out in triplicates and the results were expressed as main values $\pm$ standard error of mean. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The percentage DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) or percent inhibition = $[(Ao - A_s)/Ao] \times 100$.

Where Ao is the absorbance of control reaction and A_s is the absorbance of the test samples or standard sample (ascorbic acid).

3. RESULTS

3.1 Test for Saponins

Table 1 shows that saponin was abundant in *C. baccatum* (red), moderate in *C. Chinese* and *C. annuum* (Cayene) and trace in *C. baccatum* (yellow) and *C. annuum* (bell).

Table 1. Results of phytochemical (saponin) presence in the 5 species of *Capsicum*

1	*C. baccatum* (Yellow)	+
2	*C. baccatum* (Red)	+++
3	*C. Chinese*	++
4	*C. annuum* (Cayenne)	++
5	*C. annuum* (Bell)	+

Abundant +++; Moderate ++
Trace +; No trace -

3.2 Test for Tannins

Table 2 shows the presence of tannins was abundant in *C. annuum* (bell), moderate in *C. baccatum* (yellow), *C. baccatum* (red) and *C. chinese* and trace in *C. annuum* (cayenne).

Table 2. Results of phytochemical (Tanins) presence in the 5 species of *Capsicum*

1	*C. baccatum* (Yellow)	++
2	*C. baccatum* (Red)	++
3	*C. Chinese*	++
4	*C. annuum* (Cayenne)	+
5	*C. annuum* (Bell)	+++

Abundant +++; Moderate ++
Trace +; No trace -

3.3 Test for Flavonoids

In the test for the presence of Flavonoids, the results as shown in tables 3, 4 and 5 showed no trace of flavonoid in all the 5 species of *Capsicum* under study.

Table 3. Results of phytochemical (Flavonoids) presence in the 5 species of *Capsicum*

1	*C. baccatum* (Yellow)	-
2	*C. baccatum* (Red)	-
3	*C. Chinese*	-
4	*C. annuum* (Cayenne)	-
5	*C. annuum* (Bell)	-

Abundant +++; Moderate ++
Trace +; No trace -

3.4 Sodium Hydroxide Test

Table 4. Results of phytochemical (Flavonoids) presence in the 5 species of *Capsicum* using sodium hydroxide test

1	*C. baccatum* (Yellow)	-
2	*C. baccatum* (Red)	-
3	*C. Chinese*	-
4	*C. annuum* (Cayenne)	-
5	*C. annuum* (Bell)	-

Abundant +++; Moderate ++
Trace +; No trace -

3.5 Ammonia Test

Table 5. Results of phytochemical (Flavonoids) presence in the 5 species of *Capsicum* using ammonia test

1	*C. baccatum* (Yellow)	-
2	*C. baccatum* (Red)	-
3	*C. Chinese*	-
4	*C. annuum* (Cayenne)	-
5	*C. annuum* (Bell)	-

Abundant +++; Moderate ++
Trace +; No trace -

3.6 Test for Alkaloids

Table 6 shows that alkaloids were present in trace amounts in all the 5 species of capsicum under studies.

Table 6. Results of phytochemical (Alkaloids) presence in the 5 species of *Capsicum*

1	*C. baccatum* (Yellow)	+
2	*C. baccatum* (Red)	+
3	*C. Chinese*	+
4	*C. annuum* (Cayenne)	+
5	*C. annuum* (Bell)	+

Abundant +++; Moderate ++
Trace +; No trace -

3.7 Test for Cardiac Glycoside

3.7.1 Salkowski's test

Table 7 shows the presence of cardiac glycoside observed using salkowski's test. It was abundant in *C. baccatum* (Yellow) and *C. chinese* and moderate in *C. baccatum* (Red), *C. annuum* (cayenne) and *C. anuum* (bell).

Table 7. Results of phytochemical (Cardiac Glycoside) presence in the 5 species of *Capsicum* using Salkowski's test

1	*C. baccatum* (Yellow)	+++
2	*C. baccatum* (Red)	++
3	*C. chinese*	+++
4	*C. annuum* (Cayenne)	++
5	*C. annuum* (Bell)	++

Abundant +++; Moderate ++
Trace + No trace -

3.8 Test for Cardiac Glycosides

3.8.1 Keller-Killani test

Table 8 shows the presence of cardiac glycosides in trace amounts in all the 5 species of *Capsicum* using Keller-Killiani test.

Table 8. Results of phytochemical (Cardiac Glycoside) presence in the 5 species of *Capsicum* using Keller-killani test

1	*C. baccatum* (Yellow)	+
2	*C. baccatum* (Red)	+
3	*C. chinese*	+
4	*C. annuum* (Cayenne)	+
5	*C. annuum* (Bell)	+

Abundant +++ Moderate ++
Trace +No trace -

3.9 Test for Cardiac Glycosides

3.9.1 Lieberman's test

Table 9 shows the presence of cardiac glycosides in trace amounts in all the 5 species of *Capsicum* using Lieberman's test.

Table 9. Results of phytochemical (Cardiac Glycoside) presence in the 5 species of *Capsicum* using Lieberman's test

1	*C. baccatum* (Yellow)	+
2	*C. baccatum* (Red)	+
3	*C. chinese*	+
4	*C. annuum* (Cayenne)	+
5	*C. annuum* (Bell)	+

Abundant +++Moderate ++
Trace +No trace -

3.10 Test For Antraquinones

Table 10 shows that there was no trace of antraquinones in all the 5 species of *Capsicum* under study.

Table 10. Results of phytochemical (Antraquinones) presence in the 5 species of *Capsicum*

1	*C. baccatum* (Yellow)	-
2	*C. baccatum* (Red)	-
3	*C. chinese*	-
4	*C. annuum* (Cayenne)	-
5	*C. annuum* (Bell)	-

Abundant +++Moderate ++
Trace +No trace -

Table 11 shows the result of the DPPH scavenging activity of the different species of the 5 species of *Capsicum* species. At 20 µg/ml concentration of methanol, the mean absorbance by the species was 15% for *C. baccatum* (yellow), 11% for *C. baccatum* (red), 14% for *C. Chinese,* 11% for *C. annuum* (cayenne), 13% for *C. annuum* (bell). At 40 µg/ml concentration of methanol, the mean absorbance by the species was 22% for *C. baccatum* (yellow), 15% for *C. baccatum* (red), 19% for *C. Chinese,* 16% for *C. annuum* (cayenne), 16% for *C. annuum* (bell). At 60 µg/ml concentration of methanol, the mean absorbance by the species was 29% for *C. baccatum* (yellow), 18% for *C. baccatum* (red), 25% for *C. Chinese,* 26% for *C. annuum* (cayenne), 24% for *C. annuum* (bell). At 80 µg/ml concentration of methanol, the mean absorbance by the species was 36% for *C. baccatum* (yellow), 24% for *C. baccatum* (red), 35% for *C. Chinese,* 32% for *C. annuum* (cayenne), 31% for *C. annuum* (bell). At 60 µg/ml concentration of methanol, the mean absorbance by the species was 42% for *C. baccatum* (yellow), 31% for *C. baccatum* (red), 31% for *C. Chinese,* 38% for *C. annuum* (cayenne), 37% for *C. annuum* (bell).

Table 11. Results for DPPH Scavenging Activity of different species of pepper

Blank=0.202± 0.00

Conc. (µg/ml)	Mean absorbance					
	***C. baccatum* (Yellow)**	***C. baccatum* (Red)**	***C. Chinese***	***C. annuum* (Cayenne)**	***C. annuum* (Bell)**	**Ascorbic acid**
20	0.172 ± 0.001 (15%)	0.180 ± 0.000 (11%)	0.174 ± 0.000 (14%)	0.180 ± 0.000 (11%)	0.176 ± 0.000 (13%)	0.076 ± 0.000 (84%)
40	0.157 ± 0.000 (22%)	0.172 ± 0.002 (15%)	0.164 ± 0.001 (19%)	0.169 ± 0.001 (16%)	0.169 ± 0.001 (16%)	0.008 ± 0.000 (86%)
60	0.144 ± 0.000 (29%)	0.166 ± 0.001 (18%)	0.151 ± 0.001 (25%)	0.149 ± 0.000 (26%)	0.153 ± 0.000 (24%)	0.054 ± 0.000 (87%)
80	0.130 ± 0.000 (36%)	0.153 ± 0.001 (24%)	0.153 ± 0.001 (35%)	0.138 ± 0.000 (32%)	0.139 ± 0.000 (31%)	0.049 ± 0.000 (87%)
100	0.118 ± 0.000 (42%)	0.139 ± 0.000 (31%)	0.139 ± 0.000 (31%)	0.126 ± 0.000 (38%)	0.127 ± 0.001 (37%)	0.049 ± 0.000 (91%)

Key =values in bracket is the percentage DPPH Radial Scavenging

Table 12. Reducing power activity of different varieties of pepper

Blank=0.325± 0.000

Conc. (µg/ml)	Mean absorbance					
	C. baccatum (Yellow)	*C. baccatum* (Red)	*C. Chinese*	*C. annuum* (Cayenne)	*Capsicum annuum* (Bell)	Ascorbic acid
20	0.918 ± 0.00	0.920 ± 0.000	0.944 ± 0.000	0.941 ± 0.00	0.926 ± 0.00	0.770 ± 0.00
40	0.964 ± 0.00	0.938 ± 0.00	0.987 ± 0.000	0.958 ± 0.00	0.945 ± 0.00	1.006 ± 0.00
60	0.984 ± 0.00	0.948 ± 0.00	0.988 ± 0.00	0.963 ± 0.00	0.955 ± 0.00	1.255 ± 0.00
80	0.984 ± 0.000	0.970 ± 0.00	0.992 ± 0.00	0.978 ± 0.00	0.963 ± 0.00	1.393± 0.00
100	0.991 ± 0.000	0.992 ± 0.00	1.003 ± 0.00	0.992 ± 0.00	0.998 ± 0.00	1.755 ± 0.00

4. DISCUSSION

The results of the phytochemical analyses show important differences in composition of saponin, tannins and cardiac glycosides between in *C. baccatum* (Yellow), *C. baccatum* (Red), *C. Chinese, C. annuum* (Cayenne), *C. annuum* (Bell).

Nevertheless, the concentrations of alkaloids and anthraquinones were similar in the five species of *Capsicum* under studies. This could depend on cultivation, ripeness, storage and soil salinity, among other factors as reported by several authors [19,20,21]. The results show that flavonoids were absent in the fruit varieties of *Capsicum* under studies using different methods. The absence of flavonoids in these fruits provokes inquiries owing to the fact that flavonoids are important constituents in fruits [22]. The trace presence of Alkaloids makes these fruits non poisonous as opposed to the poisonous state of other plants with abundance of alkaloids [23]. This provides confirmatory evidence to the edibility of the fruits and its culinary uses. *C. baccatum* is abundant in saponins, this shows that it has important dietary properties, and the presence of saponin in food crops has positive implications in human health [24]. Saponins ingested as part of the human diet have been linked with a variety of effects on health, including reducing cholesterol levels [25].

Reducing power assay is one of established method for evaluation of antioxidant potential of a test sample. Basically, it involves reduction of Fe3+ into Fe2+ with the formation of Perl's Prussian blue colour complex wherein absorbance is read at 700 nm [26]. This reducing ability varies with respect to various concentrations of antioxidants present in the samples. Moreover, the reducing power ability mainly depends on the bioactive compounds including phenolics present in the test samples. Table 4 highlights the reducing power activity of the species methanolic extract of fruit stalk.

The different *Capsicum* species showed a dose dependent DPPH radical scavenging activity due to non-radical forms. The percentage inhibition of the various *Capsicum* species at the highest concentration of 100 µg/mL were as follows, *C. baccatum* yellow (42%), *Capsicum* cayenne (38%), *C. annuum* (37%) and *C. baccatum* red and *C. chinese* (31%) each. The percentage inhibition of the standard drug ascorbic acid however was 91% at 100 µg/mL. The DPPH radical scavenging activity assesses the capacity of the extract to donate hydrogen or scavenge free radicals.

However, according to literature, *Capsicum* species have been largely studied because of its commercial and medicinal values. Multiple bioactive compounds have been elucidated from variety of *Capsicum* species such as capsaicin and capsaicinoids [27]. A recent study of Zimmer *et al* reported the phenolic and flavonoid contents of fruit and seeds of *C. baccatum*, which was found to exhibit antioxidant activity in a dose dependent manner. Further, it should be noted that antioxidant potentialities showed a great variation against four antioxidant systems assayed, similar observations were reported earlier [28,29]. However, in the present study, results of the TAC, ABTS, DPPH and RPA antioxidant assays indicated a significant difference between antioxidant potential of crude methanolic extract. This phenomenon could be attributed to the various antioxidants present in the plant extracts and their differences in chemical structure which enables donation of proton to the different free radicals. Further, it is also noteworthy that other than phenolics several other potent bioactive compounds such as vitamin C, E and volatile constituents present in the test samples also scavenge free radicals [30, 31]. Nevertheless, reports also suggest the antioxidant potential of the extracts may be attributed to synergistic effect involving both phenolics and other bioactive compounds [32,33].

5. CONCLUSION

Different fruit composition of the five pepper species indicates that apart from the evident morphological differences in terms of fruit shape and appearance, they also differ in their content of saponin, tanins and cardiac glucosides and a uniform components of alkaloids and anthraquinones. The research results showed that flavonoids were absent in the fruit varieties in this study and flavonoids are reported in literature to have antioxidant potentials but this fruits have shown great antioxidant abilities with the DPPH scavenging test even with the absence of flavonoids. The trace amount of alkaloids and presence of saponins supports the usage of these fruits in the culinary industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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