Comparative Evaluation of Antioxidant Activity and Phytochemical Profile of Four *Capsicum* Fruits Species

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Summary

Capsicum fruits are commonly consumed vegetables included in foods worldwide as spices. *Capsicum* species are known to improve digestion and appetite, cure colds, coughs, fever, colic, dysentery, worms, and piles among others. This research is therefore aimed at evaluating the phytochemical as well as the antioxidant activities of four different varieties of *Capsicum* commonly consumed in Africa. The fruits were extracted using 3 different solvents; n-hexane, ethyl acetate, and methanol. The phytochemical evaluation was carried out using standard methods. Antioxidant activities were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The highest yield of extract was obtained with methanol from *Capsicum annuum* var. *grossum* (25.48%); The phytochemical screening of crude extracts revealed the presence of alkaloids, saponins, flavonoids, phenols, terpenoids, tannins, coumarins, and cardiac glycosides. The total phenolic contents of crude extracts of *Capsicum* species ranged from 25.10±0.3 to 62.01±0.1 mg GAE g^{-1} (Gallic acid equivalent) of dry weight and the flavonoid contents ranged from 206.08±0.03 to 474.23±0.04 mg QE g^{-1} (Quercetin equivalent) of dry weight. The capacity to neutralize DPPH radicals was found to be at the highest in methanolic extracts of *Capsicum* species which neutralized 50% of free radicals at the concentrations of 5.79 μg mL^{-1}, 8.08 μg mL^{-1}, 5.76 μg mL^{-1}, and 8.81 μg $mL^{-1}$for *Capsicum annuum* var. *accuminatum*, *Capsicum frutescens* var. *baccatum*, *Capsicum annuum* var. *grossum* and *Capsicum annuum* var. *abbreviatum* respectively. The study has concluded that *Capsicum* species contain phytoconstituents with high antioxidant activities and great potential to combat oxidative stress and other related diseases.

Key words

Capsicum sp., phytochemicals, antioxidant, extracts, medicinal plant

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Introduction

Foods that can effectively support health and prevent several diseases in human diets at relatively low cost are of major interest to both the scientific community and the general public. *Capsicum* is a genus of flowering plant from the family Solanaceae and is widely cultivated in South-East Asian and Latin-American countries. *Capsicum* species are cultivated as a vegetable and condiment crops worldwide (Nakhuru et al., 2018). There are five commercialized species of *Capsicum* which are: *Capsicum annuum*: (bell peppers, cayenne, jalapenos), *Capsicum frutescens*: (tabasco, Thai peppers, piri piri), *Capsicum chinense*: (habanero, Scotch bonnet), *Capsicum pubescens*: (rocoto peppers), *Capsicum baccatum*: (aji pepper) (Rêgo et al., 2011). They are eaten primarily as a food component and used as spices, flavour enhancers, vegetables, and components in herbal medicine (Chamikara et al., 2016). Compounds derived from plants have played a significant role in treating and preventing human diseases (Awolola et al., 2021; Oluwaniyi and Oladipo, 2017). They serve as important bases for new drugs and also main compounds suitable for further modification during drug development to discover new naturally occurring enzyme inhibitors (Koc et al., 2014). The enzyme inhibitors are used as agents to improve chemotherapy or to combat various diseases (Koc et al., 2014). Mateos et al. et al. (2013) report that *Capsicum* is characterized by its high levels of vitamin C (ascorbic acid), pro-vitamin A (carotene), and calcium. It is the second most consumed vegetable globally. Intakes of 50–100 g fresh pepper fruits could provide 100% and about 60% of the recommended daily amounts of vitamins C and A, respectively. The fruits have been reported to be used for weight control, curing cold, cough, dysentery and pain control (Meghwal and Goswami, 2012). They are also used traditionally for the treatment of high blood lipid and cholesterol. The presence of bioactive compounds in *Capsicum* supports the traditional medicinal use of these fruits for the treatment of different diseases. These bioactive compounds are vitamins and other secondary metabolites such as flavonoids, phenols, terpenoids, steroids and alkaloids with pharmaceutical and therapeutic applications. Chamikara et al. (2016) report that pepper is the most highly consumed spice in the world with a mean annual consumption of 3.5 million metric tons of dried fruit. Nigeria is also known to be one of the major producers of pepper in the world, accounting for about 50% of the African production. Pepper consumption in Nigeria accounts for 40% of the total vegetable consumed per day (Mohammed et al., 2016). The phytochemicals of *Capsicum* species have been evaluated, but limited studies on the phytochemical constituents of the species from Nigeria can be found in the literature. Thus, the aim of this current study is to evaluate the content, composition and antioxidant properties of phytochemicals from four varieties of *Capsicum* species commonly consumed in Nigeria.

Materials and Methods

Collection and Processing of Samples

Fully mature (red) fresh fruits of *Capsicum* species were collected from the Teaching and Research Farm, the University of Ilorin, Nigeria, where they were priorly cultivated for 4 months on natural fertile sandy-loam soil (pH 6.5-7) with constant sprinkler-drip irrigation and without application of fertilizer. The fruit samples *Capsicum annuum* var. *accuminatum* (CAVAcc), *Capsicum frutescens* var. *baccatum* (CFVBac), *Capsicum annuum* var. *grossum* (CAVGro), and *Capsicum annuum* var. *abbreviatum* (CAVAbb) were identified and authenticated at the herbarium of the Department of Plant Biology, University of Ilorin and given voucher number UILH/001/2019/ 671-674. The samples were air-dried to an average final water content of 6.50±0.2% at room temperature (27±2 °C) for 28 days; the air-dried samples were pulverized prior to extraction.

Extraction and Evaluation of Yield

Extraction was done by successive maceration of 250 g of the pulverized sample in 1000 mL of methanol (Ncube et al., 2008). The extract was decanted and filtered. The marc remaining after extraction was air dried for 24 hours and was re-extracted with 1000 mL of ethyl acetate and finally with 1000 mL of n-hexane following the same procedure as with methanol. All the extracts were concentrated using a rotary evaporator (150 ± 2 mbar and 20 ± 2 °C) and stored in opaque glass bottles prior to analysis. The percentage yield was then calculated (Terblanche et al., 2017).

Phytochemicals Screening

Qualitative phytochemical screening of the methanolic, ethyl acetate, and n-hexane extracts were carried out using standard methods: Dragendroff and Wagner test for alkaloids, a 2 mL sample of each extract was warmed with 5 mL of 2 % (v/v) H_2SO_4, heated on a water bath and filtered. A few drops of Dragendroffs' reagent were added to the filtrate and observed for the formation of an orange-red precipitate (Surendra et al., 2016); Froth test for saponins, water (10 mL) was added to 2 mL of each extract and agitated for some minutes. The mixture was observed for the formation of persistent froth (Vimalkumar et al., 2014); Keller-Killiani test for cardiac glycosides, a 2 mL sample of each extract was diluted to 5 mL with distilled water and 2 mL of glacial acetic acid was added to one drop of ferric chloride solution. This was followed by careful addition of 1 mL of concentrated sulphuric acid. The formation of a brown ring at the interface indicated a positive test (Oluwaniyi et al., 2022); NaOH for coumarins, to 2 mL of each extract was added 3 mL of 10 % (v/v) NaOH and observed for the formation of yellow colouration (Savithramma, et al., 2011); Ferric chloride for flavonoids, a portion of the powdered sample was heated with 10 mL of ethyl acetate over a steam bath for 3 min, then the mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration indicates a positive test for flavonoids (Vimalkumar et al., 2014); Salkowski test for terpenoids, to 2 mL of each extract was added 2 mL of chloroform followed by 3 mL of concentrated sulphuric acid form a layer. The formation of a reddish-brown colouration at the interface indicates the presence of terpenoid and Liberman-burchard for steroids, 2 mL of acetic anhydride was added to 1 mL extract of each sample with 2 mL H_2SO_4. Colour change from violet to green indicates the presence of steroids (Vimalkumar et al., 2014); Total phenolics in the fruit extracts: the concentrations of phenolics in the fruit extracts were determined using a spectrophotometer (VSI-502) as described by Stanković, (2010). The three extracts from each variety - methanolic, ethyl acetate and n-hexane were analyzed. The concentrations of phenolics were subsequently determined (mg/mL) from the calibration curve and expressed as Gallic acid equivalent (mg of GA g^{-1} of

extract) (Stanković, 2010); Total flavonoids in the fruit extracts: a spectrophotometer (VSI-502) was also used in the determination of the flavonoid contents of the extracts following the method of Quettier-Deleu et al. (2000). The concentration of flavonoids was extrapolated (mg mL^{-1}) from the calibration curve and expressed in terms of Quercetin equivalent (mg of Quercetin g^{-1} of extract) (Quettier-Deleu et al., 2000).

Evaluation of the Antioxidant Activity of the Fruit Extract

The ability of the plant extracts to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals was assessed by the methods reported by Kumarasamy et al., 2007 and Tekao et al., 1994. Percentage inhibition was calculated using Equation (1), while IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation of three replicates.

$$\% \text{ inhibition} = \left(\frac{\text{A of control} - \text{A of sample}}{\text{A of control}}\right) \times 100 \quad (1)$$

where 'A of control' is the absorbance of the methanolic DPPH and 'A of sample' is the absorbance of the extracts at various concentrations. Ascorbic acid was used as a reference standard.

Results and Discussion

Percentage Yield

The yield of crude extracts of the *Capsicum* species fruits CAVAcc, CFVBac, CAVGro, and CAVAbb are shown in Table 1. The highest yields were obtained from methanol extraction while the least were from n-hexane. Methanolic extracts of CAVGro and CAVAbb gave the highest yields (25.48% and 23.64% respectively) while the n-hexane extract of CFVBac gave the least yield (1.35%). The influence of solvent polarity would play a big role in the yield of the various extracts because polar compounds are easily extracted using polar solvents and vice versa (Nur Syukriah et al., 2014). The results obtained for CAVGro and CAVAbb are similar to that of Nakhuru et al. (2018) who reported 27.97% for the methanolic extract of *Capsicum assamicum*. However, there is a high degree of disparity between the results of the present study and that of Filomena et al. (2006) who reported a yield of 8.30% for the methanolic extract of *Capsicum annuum* var. *accuminatum*. The difference in sample preparation may be responsible for the observed variation.

Table 1. Percentage yield of *Capsicum* species fruits extracts

Solvent	Percentage yield (%)			
	CAVAcc	CFVBac	CAVGro	CAVAbb
n-hexane	2.02	1.35	2.50	2.88
Ethyl acetate	3.09	1.40	4.27	5.34
Methanol	16.96	19.75	25.48	23.64

Note: CAVAcc - *Capsicum annuum* var. *acuminatum*; CFVBac - *Capsicum frutescens* var. *baccatum*; CAVGro - *Capsicum annuum* var. *grossum*; CAVAbb - *Capsicum annuum* var. *abbreviatum*

Phytochemical Screening

Table 2 presents the results of various phytoconstituents such as alkaloids, saponins, flavonoids, phenols, steroids, terpenoids, tannins, and cardiac glycosides. CAVAcc appears to be the richest in phytoconstituents even though it has the lowest yield of extract. Alkaloids were found in high concentration in CAVAcc, moderate concentration in CAVGro and low concentration with CFVBac and CAVAbb. This result agreed with other results reported by Andzouana and Makomo (2016). Saponins and tannins were present in moderate concentration. Phenolics were also present in moderate concentrations in all the investigated extracts, indicating that *Capsicum* species fruits can be used as an antimicrobial agent. Flavonoids were present in high concentrations in CAVAcc and moderate concentrations in other samples analyzed. Steroids were absent, just as reported by Saidu and Garba (2011). The differences in the results obtained from the phytochemical screening of *Capsicum* species by various authors might be due to the variations in methods, geographic location, growing season, the extraction process, storage, plant part, time of collection of the plant material, climatic condition and the solvents used for the extraction. All these can influence the concentrations of phytochemicals (Zhang et al., 2015).

Phenolic Content of the Fruit Extracts of *Capsicum* Species

The Folin-Ciocalteu assay gives a crude assessment of total phenolic compounds in the fruit extracts of *Capsicum* species as shown in Fig. 1. Phenolic contents of the crude extracts ranged from 25.10 ± 0.3 to 62.01 ± 0.1 mg GAE g^{-1} of dry weight. The methanolic extract of CAVGro gave the highest phenolic content of 62.01 mg GAE g^{-1} of dry weight followed by CAVAcc (60.28 mg GAE g^{-1} of dry weight), while n-hexane extract of CAVAbb gave the lowest phenolic content of 25.10 mg GAE g^{-1} of dry weight. These results are in agreement with previous studies (Nakhuru et al., 2018; Nascimento et al., 2014) on other species. However, the results differ from the study of Shaimaa et al. (2016) that reported a range of 11.09 - 26.14 mg GAE g^{-1} of dry weight for total phenolic content for *Capsium frutescens* var. *sina* and *Capsium annuum* var. *goduion*. These researchers also de-seeded the fruits before extraction and this may also contribute to the variation in results.

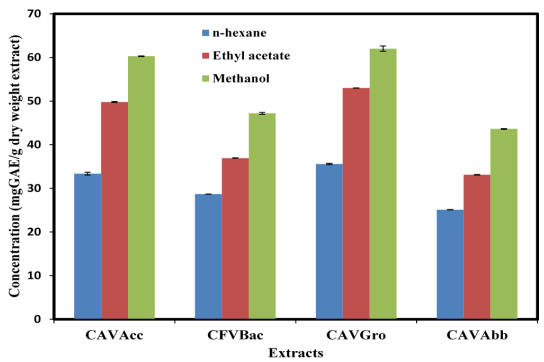

Figure 1. Phenolic content of the crude n-hexane, ethyl acetate and methanol fruit extracts of *Capsicum* species

Table 2. Phytochemical screening of the crude extracts of *Capsicum* species fruits

	CAVAcc			CFVBac			CAVGro			CAVAbb		
*PC	n-H	E. A.	M	n-H	E. A	M	n-H	E. A.	M	n-H	E. A.	M
Alkaloids	+++	+++	+++	+	+	+	++	++	++	+	+	+
Saponins	++	++	++	++	++	++	++	++	++	++	++	++
Flavonoids	+++	+++	+++	++	++	++	++	++	++	++	++	++
Steroids	–	–	–	–	–	–	–	–	–	–	–	–
Terpenoids	++	++	++	++	++	++	++	++	++	++	++	++
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	++	++	++	++	++	++	++	++	++	++	++	++
Tannis	++	++	++	++	++	++	++	++	++	++	++	++
Coumarins	+	+	+	+	+	+	+	+	+	+	+	+

Note: +++ = Present in high concentration; ++ = Present in moderate concentration; + = Present in low concentration; – = Absent; *PC = Phytoconstituents
n-H = n-Hexane; E.A = Ethyl acetate; M = Methanol

Flavonoid Concentration in the Fruit Extracts of *Capsicum* Species

From the standard Quercetin curve, the flavonoid concentration of the extracts was calculated (y = 0.0038x + 0.0209) and values obtained ranged from 206.08 ± 0.03 to 474.23 ± 0.04 mg QE g^{-1} (Fig. 2). The results of the flavonoid concentration of the extracts are presented in Fig. 2. Methanolic extract of CAVGro gave the highest flavonoids content of 474.23 ± 0.04 mg QE g^{-1} while n-hexane extracts have the lowest flavonoids contents with CAVAbb (206.08 ± 0.03 mg QE g^{-1}) being the lowest. Ethyl acetate, the medium polar solvent showed medium flavonoid concentration (Fig. 2). The flavonoid contents of the plant are both concentration and polarity dependent i.e. the higher the polarity and concentration, the higher the flavonoid concentration in the extracts (Jing et al., 2015). Several studies have confirmed the presence of flavonoids in *Capsicum* fruits. Blanco-Ríos et al. (2013) reported that red peppers generally exceeded green peppers in flavonoid content, which significantly contributes to the antioxidant power of the *Capsicum* fruit.

The result of this research is in agreement with Soare et al. (2017) who reported a flavonoid concentration range of 292 – 480 mg QE g^{-1} in another species analysed. The results obtained also indicate that the phytochemical composition of *Capsicum* species is variety-dependent.

Antioxidant Assay

DPPH is a fast, reliable, simple and preferred technique employed for determining the antioxidant potential of a wide collection of test samples (Irshad et al., 2012). DPPH is widely used to test the ability of compounds to scavenge free radicals or act as hydrogen donors, and thus evaluate antioxidant activity (Tailor and Goyal, 2014).

The absorbance of ascorbic acid, which was used as standard, was 1.037 and this was used to calculate the percentage inhibition of the extracts using Equation 1. The IC_{50} values, obtained from a plot of % inhibition versus concentration, using a non-linear regression algorithm are presented in Fig. 3 to 6.

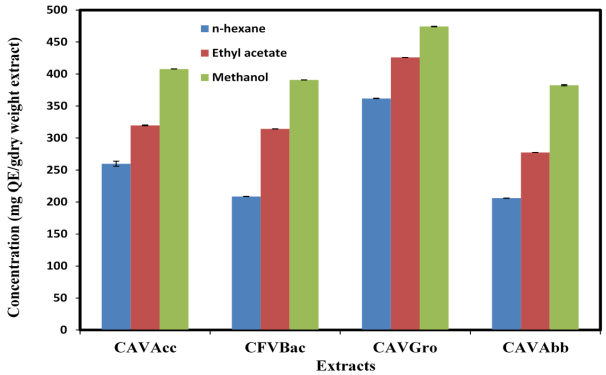

Figure 2. Flavonoid content of the crude n-hexane, ethyl acetate and methanol fruit extracts of *Capsicum* species

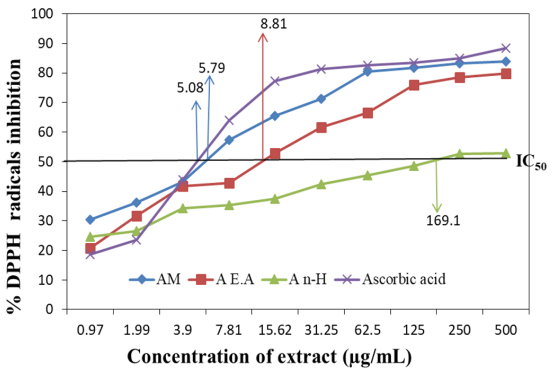

Figure 3. Inhibition (%) of DPPH radical versus concentration of *Capsicum annuum* var. *acuminatum*

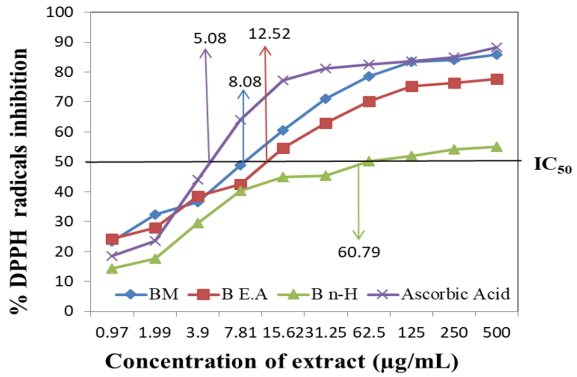

Figure 4. Inhibition (%) of DPPH radical versus concentration of *Capsicum frutescens* var. *baccatum*

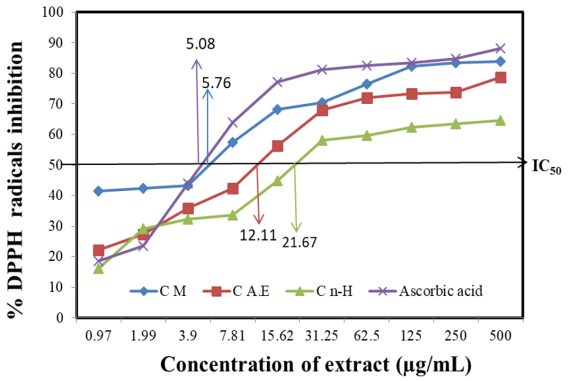

Figure 5. Inhibition (%) of DPPH radical versus concentration of fruit extracts of *Capsicum annuum* var. *grossum*

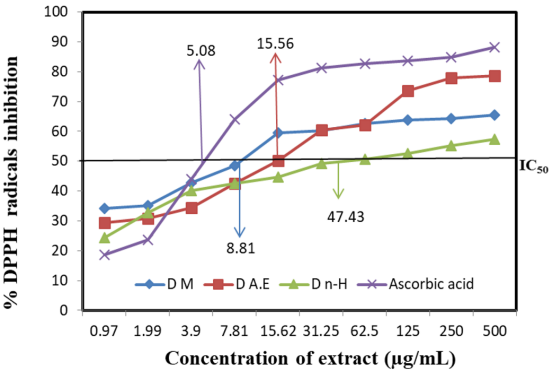

Figure 6. Inhibition (%) of DPPH radical versus concentration of *Capsicum annuum* var. *abbreviatum*

The capacity to neutralize DPPH radicals was found to be the highest in the methanolic extracts, which neutralized 50 % of free radicals at concentrations of 5.79 µg mL^{-1}, 8.08 µg mL^{-1}, 5.76 µg mL^{-1}, and 8.81 µg mL^{-1} for CAVAcc, CFVBac, CAVGro, and CAVAbb respectively, compared with 5.08 µg mL^{-1} recorded for ascorbic acid. The lowest activities were obtained for the n-hexane extracts. Methanolic extracts of CAVAcc and CAVAbb manifested comparable capacity for neutralization of DPPH radicals with ascorbic acid. Nakhuru et al. (2018) reported IC_{50}=5.45-74.42 µg mL^{-1} for *Capsicum assamicum* while Gheith and El-Mahmoudy (2017) reported IC_{50}=113 µg mL^{-1} for ethanolic extract of *Capsicum annuum*. The extracts that present the highest antioxidant activity (Fig. 3, 4, 5, and 6) have the highest concentration of phenolics and flavonoids (Fig. 1 and 2). Therefore, the antioxidant property of the extracts can be attributed to the presence of polyphenolic compounds like flavonoids. It has been reported that radical scavenging activity is mainly due to the presence of hydroxyl groups in aromatic rings of phenolics (Prabakaran et al., 2017). These results suggest that phenolic acids and flavonoids may be the major contributors to the antioxidant activity of *Capsicum* species.

Conclusion

Phytochemicals, antioxidants, and percentage yields of extracted *Capsicum* species fruits were evaluated. The highest yield and concentration of compounds in the *Capsicum* species extracts were obtained using methanol. Among the methanolic extracts, the highest yields were from *Capsicum annuum* var. *grossum* of methanolic extract. The highest antioxidant was found in methanolic extracts. The findings of the present study suggest that *Capsicum* species of Nigerian origin are a good source of natural antioxidants of high importance as a therapeutic agent in combating or slowing the progress of aging and age-associated oxidative stress-related and can also be used in phytotherapy.

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