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Comparative Assessment of Proximate and Phytochemical Composition and Antioxidant, Anti-diabetic and Anti-Inflammatory Properties of Pericarp and Seeds of *Capsicum annum* L.

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Abstract

This study evaluates the proximate and phytochemical composition and antioxidant, anti-diabetic, and anti-inflammatory properties of *Capsicum annum* L. seed powder (CSP) and pericarp powder (CPP). The CSP and CPP were analyzed for moisture, crude protein, ash, crude fat, crude fiber, nitrogen-free extract, flavonoids, phenol, tannins, alkaloids, saponins, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), ferric ion reducing antioxidant power (FRAP), alpha-amylase, alpha-glucosidase, lipase and albumin inhibitory properties and anti-proteinase properties. The moisture, crude protein, ash and crude fat concentrations were significantly higher in CPP compared to CSP; while the concentrations of crude fiber and nitrogen-free extracts were significantly lower in CSP than CPP. The phenol, alkaloids and saponins concentrations of the CPP were significantly higher than CSP; while the flavonoids and steroid concentrations were higher in CPP compared to CSP. The vitamin C, DPPH, ABTS, FRAP and lipid peroxidation inhibition of CPP was significantly higher than CSP. The alpha-amylase inhibition and alpha-glucosidase inhibition of CPP were significantly higher than in CSP. The lipase inhibitory and anti-proteinase properties of CSP were higher than CPP; while the albumin inhibitory property was higher in CPP than in CSP. In conclusion, CPP offers better protein, minerals, and fat content compared to CSP with stronger antioxidant, anti-cholesterol, and anti-diabetic properties.

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Introduction

Utilizing nutraceuticals has been demonstrated to be able to both prevent and treat several diseases [1, 2]. Particularly, numerous medical conditions can be treated with the help of nutritional supplements, including arthritis, colds, coughs, cancer, digestion problems, insomnia, depression, high cholesterol, diabetes, and pain relief [3-5]. Due to their broad availability, chemical makeup and potential medical benefits, botanicals and their preparations are becoming more and more well-known and commonly accepted as a staple of people's and animals' regular meals [6]. There are suggestions that some foods, such as those flavored with spices and herbs have the propensity to lower the chance of developing diseases and can significantly enhance the quality of life [7, 8].

Peppers are herbaceous or suffruticose plants of 30 to 200 cm in height with whitish or rarely purplish flowers. The fruit is a berry with many seeds that are variable in shape, size and color. Sweet peppers can present as square or rounded fruits while spicy varieties exhibit elongated and conical fruits [9]. Peppers are beneficial for health since they contain a wealth of phytonutrients and have anti-inflammatory and antioxidant qualities [9]. It is well recognized that *Capsicum species* are abundant in substances including capsaicinoids, capsinoids, carotenoids, flavonoids, vitamins, essential oils, and other phytochemicals, which have a distinctive flavor, fragrant qualities and health advantages [10]. According to research, consuming anti-inflammatory nutrients can lower the resurgence of chronic inflammation, while antioxidant nutrients can lessen cellular oxidative stress [11]. Consequently, peppers could act as a nutraceutical feed additive in both human and animal nutrition since they are a rich source of phytonutrients and possess antioxidant and anti-inflammatory characteristics [12].

The medicinal and nutraceutical qualities of botanicals may be influenced by their chemical makeup [6]. Plants absorb essential nutrients from their environment and the accumulation of these nutrients varies in different parts of the plants [13]. For instance, the location and relative abundance of metabolites and their precursors in different parts of the chili pepper fruit, such as the pericarp, placenta and seed, remains unclear. For instance, it is still unknown where metabolites and their precursors are located and how abundant they are in various chili pepper fruit components [10]. As a result, further research is required to characterize the bioactive content profile of different parts of botanicals, especially as dietary supplements are referred to as nutraceuticals when they are utilized for health-related rather than dietary objectives [8]. Therefore, the goal of this study is to compare and evaluate the phytochemical content and antioxidant, anti-diabetic, and anti-inflammatory effects of the seed and pericarp of *Capsicum annum* L. fruit.

Materials and Methods

Capsicum annum L fruits parts and processing

Whole fresh *C. annum* fruits were purchased from farmers in Akure, Nigeria. The *C. annum* fruits were authenticated by a crop scientist from the Department of Agronomy, Adekunle Ajasin University (AAUA), Akungba Akoko, Nigeria. The *C. annum* fruits were sliced with a clean stainless knife. Thereafter, the seeds and the pericarp of the pepper fruits were separated carefully, spread lightly on a stainless tray and air-dried for 14 days. On the fifteenth day, the *C. annum* fruits' pericarp and seeds were sun dried for 2 hours and powdered with a blender to form *C. annum* fruit pericarp powder (CPP) and *C. annum* fruit seed powder (CSP), respectively. The CPP and CSP were stored in plastic rubber in the freezer for analysis. Five grams (5 g) of each sample (CPP and CSP) were placed within separate glass thimbles and subjected to extraction using 200 ml of distilled water as the extraction solvent. The mixture was heated on a hot plate at 30-40ºC and mixed with continuous stirring for 20 minutes. Following the extraction, the resulting extracts were passed through Whatman qualitative filter paper No. 1 for filtration. After filtration, the extracts were concentrated to a state of dryness. This concentration process was carried out using a rotary evaporator. The concentrated extracts were then stored in a refrigerator set at 4°C, awaiting further analysis.

Proximate and phytochemical analysis

The CPP and CSP were analyzed for moisture, crude protein, ash, crude fat, crude fiber, and nitrogen-free extract using the AOAC methods [14]. Chemicals and reagents for chemical analysis were all bought from Sigma-Aldrich. In this experiment, only analytical reagent-grade chemicals were employed. The concentrations of flavonoids [15], phenol [16], tannins [17], alkaloids [18], and saponins [19] in CPP and CSP were determined using standard procedures outlined by Oloruntola [20]. To determine the steroid [21] concentration in CPP and CSP, 1 ml of sample extract was put into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5

% w/v, 2 ml) were then added after the potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water bath for 30 minutes at 70±2ºC with intermittent shaking before being diluted with distilled water to the required concentration. At 780 nm, the absorbance was computed and compared with a blank for the reagent [21].

Antioxidant properties analysis

The procedures used for the determination of vitamin C [22], 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [16], 2, 2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) [23], Ferric ion reducing antioxidant power (FRAP) [24], and lipid peroxidation inhibition [25], were recently published by Oloruntola [20] and Oloruntola et al. [8].

Alpha-amylase and alpha-glucosidase inhibition properties

The procedures published by Oloruntola and Ayodele [6] were used for the determination of alpha-amylase [26] and alpha-glucosidase [27] inhibition properties.

Lipase and albumin inhibition properties and anti-proteinase properties

With some slight adjustments, the method published by Nakai et al. [28] was used to evaluate the activity of porcine pancreatic lipase (type II, from porcine pancreas). Twenty milligrams (20 mg) of the samples were dissolved in 4 ml of distilled water containing 2% dimethyl sulfoxide (DMSO) to obtain 5 mg/ml stock solutions. This solution was then diluted using 13 mM Tris-HCl buffer, which has 150 mM NaCl and 1.3 mM $CaCl_2$ (pH 8.0) and a final DMSO content of 2.5% v/v. As a substrate, 4-methylumbelliferyl oleate was employed. The buffer stated above was added right before usage, and both the substrate and the enzyme were diluted. In black microtiter plates, an aliquot of 25 µl of the test solution and 50 µl of 0.1 mM 4-MU solution was combined, and then 25 µl of 0.2 mg/ml enzyme solution was added to each well to initiate the reaction. The reaction was stopped by adding 100 µl of 0.1 M citrate buffer (pH 4.2) after 30 minutes of incubation at 37°C. Using a multi-label counter (PerkinElmer 2030 ARVO X4; PerkinElmer Life and Analytical Sciences), the fluorescence associated with the enzymatically produced 4-methylumbelliferone product was observed at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. As a reference substance, Orlistat, a well-known inhibitor of pancreatic lipase, was used. Before the enzymatic reaction, a stop solution was added to assess the activity of the negative control. The following formula was used to determine the inhibitory activity:

$$\text{Inhibition (\%)} = [(FES_1 - FES_2) - (FES_3 - FES_4)] / (FES_1 - FES_2) \times 100$$

where FES_1 represents the fluorescence of an enzyme and substrate without the test material (after adding stop solution after enzymatic reaction); FES_2 is a fluorescence assay that lacks the test substance but includes an enzyme and a substrate (after adding stop solution before enzymatic reaction); the fluorescence with an enzyme, a substrate, and test material is FES_3 (after adding stop solution after enzymatic reaction); and the fluorescence with an enzyme, a substrate, and test material is FES_4 (after adding stop solution before enzymatic reaction). The concentration needed to inhibit 50% of pancreatic lipase activity is indicated by the IC_{50} value.

The albumin inhibition and anti-proteinase properties assays were carried out as outlined by Osman et al. [29] and Rajesh et al., [30], respectively, and recently published by Oloruntola et al. [8].

Statistical analysis

The results were obtained by calculating the average of triplicate measurements for each data point. The data analysis was performed using the SPSS version 20 statistical software [2]. To assess significant variations in mean values, a one-way analysis of variance (ANOVA) was employed.

Results

The moisture, crude protein, ash, and crude fat concentrations were significantly higher in CPP, compared to CSP; while the concentrations of crude fiber and nitrogen-free extracts were significantly (P=0.01) lower in CSP than CPP (Fig. 1). Table 1 shows the phytochemical composition of *C. annum* fruits' pericarp powder (CPP) and *C. annum* fruits' seed powder (CSP). The phenol, alkaloids and saponins concentrations of the CPP were significantly (P=0.01) higher than CSP; while the flavonoids (P=0.09) and steroid (P=0.05) concentrations tend to be higher in CPP, compared to CSP. The vitamin C, DPPH, ABTS, FRAP and lipid peroxidation inhibition of CPP was significantly (P=0.01) higher than CSP (Table 2). Fig. 2 shows that the alpha-amylase inhibition and alpha-glucosidase inhibition of CPP were significantly (P=0.002; 0.001, respectively) higher than in CSP. The lipase

Table 1 Phytochemical composition of *Capsicum annum* seeds and pericarp.

Fruit part	Flavonoid (mg/g)	Phenol (mg/g)	Tannins (mg/g)	Alkaloids (%)	Saponins (mg/g)	Steroids (mg/g)
Pericarp	7.45	61.59[a]	0.19	28.50[a]	34.49[a]	3.02
Seed	6.91	44.68[b]	0.20	21.05[b]	29.79[b]	2.39
SEM	0.16	4.05	0.01	1.68	1.06	0.17
P-value	0.09	0.01	0.74	0.01	0.01	0.05

[ab] Means with different superscripts within a column are significant.
SEM: standard error of the means

Table 2 The antioxidant properties of *Capsicum annum* seed and pericarp

Fruit part	Vitamin C (mg/g)	DPPH (%)	ABTS (%)	FRAP (mg/g)	LPI (%)
Pericarp	14.18[a]	65.97[a]	79.53[a]	22.23[a]	61.93[a]
Seed	7.77[b]	43.42[b]	69.91[b]	15.05[b]	38.56[b]
SEM	1.45	5.05	2.16	1.67	5.26
P value	0.01	0.01	0.01	0.01	0.01

[ab] Means with different superscripts within a column are significant.
SEM: Standard error of the means; DPPH: 2,2-diphenyl-1-picrylhydrazyl hydrate; ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid; FRAP: Ferric ion reducing antioxidant power; LPI: lipid peroxidation inhibition

inhibitory and anti-proteinase properties of CSP were higher (*P*=0.001) than CPP; while the albumin inhibitory property was higher in CPP (*P*=0.001) than in CSP (Fig. 3).

Discussion

The proximate analysis unveils the estimated quantities of moisture, crude protein, total fat, total carbohydrate and dietary fiber in feed or food [31]. To prevent the growth of microorganisms like fungi and mold, the level of moisture content is one of the key parameters in storage [31]. Therefore, the CPP, when used as an additive in animal feed could increase its moisture content, when compared to CSP and consequently deter the storage span of the feed. Although the moisture contents of 16.7% and 12.1% were recorded in this study for CSP and CPP, respectively, outside and below 16%-46% moisture concentration were found as the favorable range for microbial development, depending on the microorganism and feed material or substrate [32]. Interestingly, the elevated crude protein, ash and crude fat CPP could provide higher dietary protein [33], minerals and fatty acids, oil-soluble dyes, fat-soluble vitamins, and steroids [31] when used as a feed supplement, compared to CSP. Dietary fiber must be consumed in minimum amounts to maintain the gut's proper physiological function [34]. Therefore, the CSP because of its relatively higher crude fiber content, when used as a supplement could promote the normal physiological function of the gut of both man and animals, when compared to CPP. In addition, the higher nitrogen-free extract of CSP, compared to CPP shows that it could contribute more dietary energy to human and animal nutrition [6].

The CPP appears to possess superior nutraceutical values when compared to CSP in this study due to its higher concentration of phenol, alkaloids and saponins concentrations. Due to their antioxidant qualities and possible health benefits, phenolic chemicals, which are present in all plants and are vital to both human and animal diets, are of great interest. Various phenolic chemicals included in food or feed may reduce the incidence of health issues due to their antioxidant action, according to the growing body of research [35]. In addition, antioxidants prevent the growth of rancidity, delay the generation of hazardous oxidation products, maintain nutritional quality, and lengthen product shelf lives when added to foods [35]. Therefore, the CSP and CPP, when used as a dietary supplement could provide some health benefits and also improve the feed or food shelf life.

Alkaloids make up about 20% of the known secondary metabolites present in plants and are crucial to an organism's natural defense [36]. Alkaloids are particularly well known for their therapeutic uses as anesthetics, cardioprotectants, and anti-inflammatory drugs. In clinical contexts, well-known alkaloids including morphine, quinine, ephedrine, strychnine, and nicotine are employed [37]. Therefore, by implication, dietarily supplemented, CPP will provide better nutraceutical effects in their consumer, when compared to CSP. Saponins lessen cancer risk, lower blood cholesterol levels, and moderate the response of blood sugar. A high-saponin diet can be used to cure hypercalciuria,

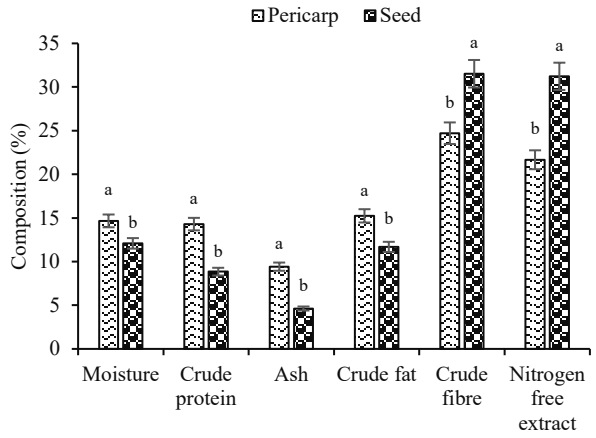

Fig. 1 Proximate composition of *Capsicum annum* seeds and pericarp. Bars with different English letters are significantly different at $P<0.05$.

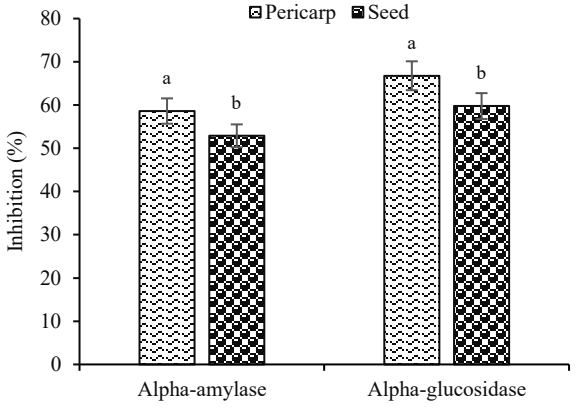

Fig. 2 Alpha-amylase inhibition and alpha-glucosidase inhibition of *Capsicum annum* seeds and pericarp. Bars with different English letters are significantly different at $P<0.05$.

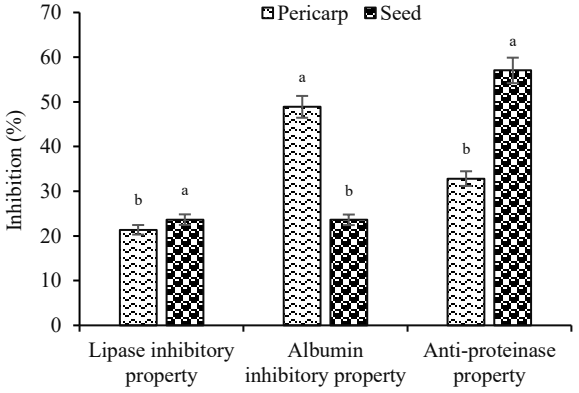

Fig. 3 Lipase inhibitory property, albumin inhibitory property and anti-proteinase property of *Capsicum annum* seeds and pericarp. Bars with different English letters are significantly different at $P<0.05$.

prevent dental decay and platelet aggregation, as an antidote for acute lead poisoning, and treat hypercalciuria [38]. In addition, saponins act as immunological adjuvants by enhancing the immune response to antigens [39]. As a result, CPP will have greater hypocholesterolemic, immunomodulatory, and anti-poisoning benefits than CSP when taken as a dietary supplement due to the considerably higher saponins concentration seen in CPP in this study.

A vast variety of foods and medicinal plants contain natural antioxidants. Exogenous antioxidants are mostly produced from food and medicinal plants, including fruits, vegetables, cereals, mushrooms, drinks, flowers, spices, and traditional medicinal herbs [40]. The content of vitamin C, DPPH, ABTS, FRAP and the lipid peroxidation inhibitory effects of CPP and CSP suggest that these supplements may be potential natural antioxidant feed supplements or ingredients suitable for animal or human nutrition. Proteins and lipid membranes are guarded against oxidative damage by the first line of antioxidant defense, which includes vitamin C. In addition, as a free radical neutralizer and free radical damage preventer, vitamin C can act both within and outside of cells [41]. The DPPH determination was described in a publication as an appropriate approach for assessing the antioxidant capacities of feed or food [42]. The ABTS [43], lipid peroxidation inhibition [44] and FRAP [45] assays also reveal the antioxidant activities of foods [43, 35]. As earlier reported, the concentration of bioactive compounds varies in all plant organs or plant parts such as fruits, leaves, roots, tubers, and barks as well as the whole plant [46]. Given that CPP had higher levels of vitamin C, DPPH, ABTS, FRAP and lipid peroxidation inhibition than CSP in this investigation, it is likely that CPP has superior antioxidant capabilities.

Numerous herbal extracts are being utilized to treat diabetes because they have been shown to have anti-diabetic properties. Due to the paucity of consistent scientific data, many of these therapeutic plants have not; however, achieved significant prominence as medications [47]. The CSP and CPP were found to have the potential to inhibit α-amylase and α-glucosidase in the current study. In particular, when compared to CSP, the CPP could be considered a superior natural α-amylase and α-glucosidase inhibitor of herbal origin that could regulate post-prandial hyperglycemia by lowering the glucose release from starch and delaying carbohydrate absorption by inhibiting the activity of carbohydrate hydrolyzing enzymes in the small intestine and subsequently control diabetes [48, 49]. The ability of

botanicals to inhibit pancreatic lipase and their potential application as anti-obesity medicines are both now being studied [50]. This study shows that CSP has superior lipase inhibitory properties compared to CPP. This could be linked to the relatively higher crude fiber content of CSP (31.5%) over that of CPP (24.7%). As previously reported, dietary fibers could have an inhibitory effect on pancreatic lipase [51].

The basis for considering medicinal plants for the treatment of distressing and complex biological disorders like inflammation is their bioactive compounds, which include flavonoids, alkaloids, saponins, coumarins, and glycosides [52]. It is usual practice to gauge how effectively plant parts reduce inflammation *in vitro* by measuring their albumin inhibitory and anti-proteinase properties [53]. According to this study, the distribution and concentration of the bioactive chemicals in plant parts differ as evidenced by the higher albumin inhibitory property demonstrated by CPP as compared to CSP (48.9% vs. 23.6%) and stronger anti-proteinase properties exhibited by CSP as compared to CPP (57.4% vs. 32.8%) [13]. However, by preventing albumin or protein denaturation [53], CSP and CPP both showed some degree of anti-inflammation inhibitory capabilities. Black pepper [54] and the leaf and roots of *Euphorbia hirta* [53] have been found to have anti-inflammatory properties *in vitro*.

Conclusions

Finally, CPP could be a better source of crude protein, minerals and fat, compared to CSP, while CSP provides higher dietary fiber and nitrogen-free extract. The CPP could have stronger antioxidant, anti-cholesterol and antidiabetic properties, compared to CSP. The anti-inflammatory activities are distributed across CSP and CPP.

Conflict of interest

The authors declare no conflict of interest.

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