

Distribution of Metabolites and Antioxidant Activity in the Berry and Shoot of *Solanum pseudocapsicum* Grown in Nigeria

ABSTRACT

Aim: To evaluate the distribution of metabolites and *in vitro* antioxidant activity in the berry and shoot of *Solanum pseudocapsicum* (*S. pseudocapsicum*).

Study design: Berry and shoot samples of *S. pseudocapsicum* were randomly collected from different plants of the species in the same natural habitat, and were subsequently processed and used for the spectrophotometric quantification of their metabolites and *in vitro* antioxidant activity.

Methodology: The samples were oven-dried to a constant weight, and were later milled separately into a fine particle size powder. The milled samples were extracted with methanol, and the extracts were analyzed for their metabolites content and *in vitro* antioxidant activity using appropriate methods.

Results: The berry had significantly ($P < 0.05$) higher total protein, total lipid and amylose levels than the shoot. Conversely, the shoot had significantly ($P < 0.05$) higher starch, total soluble sugar, amylopectin, and vitamin C contents than the berry. Secondary metabolites (total phenol, tannin, total flavonoid, total saponin and total alkaloid) were consistently more predominant in the berry than in the shoot, except total carotenoid. Methanol extracts of both the berry and the shoot displayed antioxidant activity *in vitro*, with the berry having significantly ($P < 0.05$) higher ABTS radical scavenging ability and ferric reducing antioxidant power (FRAP) than the shoot. Both extracts also scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical in a dose-dependent manner; with the IC_{50} of the shoot (1.88 mg/mL) being lower than that of the berry (2.33 mg/mL).

Conclusion: The distribution of metabolites and antioxidant activity in the berry and the shoot of *S. pseudocapsicum* provide support for their medicinal uses. However, the preponderance of secondary metabolites and antioxidant activity in the berry than in the shoot might confer it with more potential to ameliorate oxidative stress in disease conditions, than the shoot.

Keywords: *Solanum pseudocapsicum*; Plant metabolites; Antioxidant activity; Oxidative stress.

1. INTRODUCTION

Plant cells produce two types of metabolites, namely, primary and secondary metabolites. Primary metabolites including carbohydrates, lipids, proteins and organic acids are produced as a result of photosynthesis, and are directly involved in growth and metabolism. On the other hand, secondary metabolites such as alkaloids, phenolics, essential oils, terpenes, sterols, flavonoids, lignins and tannins, are considered products of primary metabolism, and are generally not involved in metabolic activity [1]; rather they protect plants against herbivores and microorganisms, attract pollinators and seed-dispersing animals, and act as signal molecules under stress conditions [2, 3]. Secondary metabolites, otherwise

known as phytochemicals, have been linked to many positive effects in disease conditions such as coronary heart diseases, diabetes, high blood pressure, cataracts, degenerative diseases, and obesity [4, 5]. They are also the major source of pharmaceuticals, food additives, fragrances and pesticides [6].

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability [7]. Furthermore, novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties [7]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major sources of oxidative stress in cells, damaging proteins, lipids, and DNA [8]. Oxidative DNA damage has been implicated as a cause of cancer [9], aging and neurodegenerative diseases such as Alzheimer's and Parkinson's [10, 11], cardiovascular diseases such as arteriosclerosis [12]. Therefore, prevention of oxidative stress caused by ROS and RNS has important implications for the prevention and treatment of disease.

There is increasing evidence that weeds such as *Solanum pseudocapsicum* are relatively high in bioactive secondary compounds which are likely to hold promise for drug discovery [13]. *S. pseudocapsicum* L. (*Solanaceae*) is an erect and highly branched shrub with non-spiny stem reaching a height of five meters. It is often cultivated as an indoor ornamental plant due to its beautiful berries. At maturity, the berries are attractive having red to yellow coloration. The number of seeds per berry ranges from 50 to 100 while the berries could be as many as 100 per plant [14]. The berries contain solanocapsine and other minor alkaloids [15]. The medicinal values of *S. pseudocapsicum* have been reported to include hepatoprotective [16]; antispasmodic, cytotoxic and antihypertensive [17]; antimicrobial [18]; and antitumor [19].

In view of the medicinal values of *S. pseudocapsicum*, this study was designed to evaluate the distribution of metabolites and antioxidant activity in the berry and shoot of *S. pseudocapsicum* grown in a natural habitat in Nigeria.

2. MATERIALS AND METHODS

2.1 Samples collection and preparation

About 20 g of berry and shoot samples of *S. pseudocapsicum* were randomly collected from different plants of the species in the same natural habitat in Akingbile area of Moniya, Ibadan, Nigeria, and were later authenticated at the Department of Botany, University of Ibadan, Nigeria. Samples were then sorted, rinsed with distilled water and oven-dried at 50 °C to a constant moisture content of about 5%. The dried samples were subsequently milled to a fine particle size (0.5 mm), packed in air-tight plastic vials and stored at 4°C until analysis.

2.1.1 Preparation of Methanol extract: 2 g of sample was extracted by steeping in 100 mL of methanol overnight, after which it was centrifuged at 3,000 rpm for 10 min. The supernatant, subsequently referred to as methanol extract, was collected and stored at -4 °C for further analysis.

2.2 Determination of Primary Metabolites: The primary metabolites (protein, total lipid, starch, total soluble sugar, vitamin C, amylose and amylopectin) levels were quantified using appropriate

methods as follows: protein and lipid [20]; starch and total soluble sugar [21]; and Vitamin C [22]. The amylose content was determined following the method of AACC [23], while the amylopectin was calculated by difference [24] using the following formula:

$$\text{Amylopectin (\%)} = 100\% - \text{amylose (\%)}$$

2.3 Determination of Secondary Metabolites

Total phenol: The total phenol content of the extracts was determined according to the Folin–Ciocalteu method reported by Chan *et al.* [25], and was expressed as gallic acid equivalent (GAE) in mg/g sample.

Tannin: The tannin content of the extracts was determined according to the method reported by Amorim *et al.* [26], and was expressed as tannic acid equivalent (TAE) in mg/g sample.

Total flavonoid: The total flavonoid content, expressed as quercetin equivalent (QE) in mg/g sample, was determined using aluminum chloride method as reported by Kale *et al.* [27].

Total carotenoid: The total carotenoid content of the samples was determined according the method of Rodriguez-Amaya [28]. The total carotenoid was separated from other pigments in the ether extract by passing it through an alumina (Activity III) column, and eluting the total carotenoid with ether to a final volume of 25 mL. To avoid the degradation of the total carotenoid, analysis was carried out under subdued light and the extract was collected into a vial wrapped with aluminum foil.

Total saponin: The total saponin, expressed as diosgenin equivalent in mg/g of sample, was determined by the method described by Makkar *et al.* [29].

Total alkaloid: The total alkaloid content of the samples was measured using 1,10-phenanthroline method described by Singh *et al.* [30].

2.4 Determination of antioxidant activity

ABTS radical-scavenging ability: The ABTS^{*} scavenging ability of both extracts were determined according to the method described by Sellappan and Akoh [31]. The ABTS^{*} was generated by incubating equal volume of a 7 mM ABTS aqueous solution with K₂S₂O₈ (2.45 mM) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 mL appropriate dilution of the extract was added to 2.0 mL ABTS^{*} solution and the absorbance was measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

DPPH free radical scavenging ability: The DPPH free radical scavenging ability of the extracts was determined as described by Cervato *et al.* [32]. Briefly, appropriate dilution of the extracts (1 mL) was mixed with 3 mL of 60µM methanol solution of DPPH radicals; the mixture was left in the dark for 30 min before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH^{*} on addition of test samples in relation to the control was used to calculate the percentage scavenging ability, following the equation:

$$\% \text{ scavenging ability} = (A_{517_{\text{control}}} - A_{517_{\text{sample}}}) \div A_{517_{\text{control}}} \times 100.$$

The IC₅₀ was calculated from the % scavenging ability versus extract concentration non-linear regression curve.

Ferric reducing antioxidant power: Reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu [33]. 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. Then 5 mL supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

2.5 Statistical analysis: Experimental results were reported as mean ± standard deviation of parallel triplicate measurements. Analysis of variance (ANOVA) was carried out on the result data at 95% confidence level using SPSS statistical software package (version 17).

3. RESULTS

The result of the primary metabolites distribution in the berry and the shoot of *S. pseudocapsicum* is presented in Table 1. The berry had significantly ($P < 0.05$) higher total protein, lipids and amylose levels than the shoot. Conversely, the shoot had significantly ($P < 0.05$) higher starch, total soluble sugar, amylopectin and vitamin C contents than the berry. The result further revealed that amylopectin was more abundant than amylose in both the berry and the shoot.

Table 1. Distribution of primary metabolites in the berry and shoot of *S. pseudocapsicum* on dry weight basis.

Metabolite	Berry	Shoot
Protein (%)	18.23 ± 0.25 ^a	10.64 ± 0.25 ^b
Total Lipid (%)	8.20 ± 0.03 ^a	1.16 ± 0.01 ^b
Starch (%)	15.20 ± 0.16 ^b	25.49 ± 0.61 ^a
Total soluble sugar (%)	9.63 ± 0.14 ^b	11.33 ± 0.10 ^a
Amylose (%)	1.02 ± 0.04 ^a	0.11 ± 0.01 ^b
Amylopectin (%)	99.01 ± 0.07 ^b	99.90 ± 0.01 ^a
Vitamin C (mg/g)	1.55 ± 0.03 ^b	1.91 ± 0.02 ^a

Data represent the mean ± standard deviation of triplicate readings. Values with different lowercase superscript letter along the same row are significantly different ($P < 0.05$).

The result of the secondary metabolites distribution in the berry and the shoot of *S. pseudocapsicum*, presented in Table 2, showed that the berry had significantly ($P < 0.05$) higher total phenol, tannin, total flavonoid, total saponin and total alkaloid than the shoot. On the other hand, the total carotenoid content of the shoot was significantly ($P < 0.05$) higher than that of the berry.

Table 2. Distribution of secondary metabolites in the berry and shoot *S. pseudocapsicum* on dry weight basis

Metabolite	Berry	Shoot
Total phenol (mg/g)	7.02 ± 0.07 ^a	5.01 ± 0.05 ^b
Tannin (mg/g)	7.97 ± 0.13 ^a	5.87 ± 0.13 ^b
Total flavonoid (mg/g)	0.45 ± 0.03 ^a	0.31 ± 0.01 ^b
Total carotenoid (µg/g)	9.92 ± 0.14 ^b	21.39 ± 0.18 ^a
Total saponin (mg/g)	44.73 ± 0.07 ^a	20.11 ± 0.27 ^b
Total alkaloid (mg/g)	29.03 ± 0.92 ^a	18.12 ± 0.11 ^b

Data represent the mean ± standard deviation of triplicate readings. Values with the different lowercase superscript letter along the same row are significantly different (P < 0.05).

The antioxidant activity of the berry and the shoot extracts, determined by their ability to scavenge DPPH and ABTS free radicals, and reduce Fe³⁺ to Fe²⁺, is presented in Table 3. Both extracts exhibited DPPH free radical-scavenging activity in a dose-dependent manner, as depicted by their % scavenging ability-extract concentration curves (Figure 1). However, their IC₅₀ values (2.33 mg/mL and 1.88 mg/mL, for the berry and the shoot respectively), were higher than that of vitamin C (0.04 mg/mL), that was used as a reference antioxidant. Furthermore, the ABTS* scavenging ability of the extracts, reported as trolox equivalent antioxidant capacity (TEAC), revealed that the ability of the berry to scavenge ABTS* was significantly (P < 0.05) higher than that of shoot. The FRAP of the extracts, reported in gallic acid equivalent (GAE/g), revealed that the berry had a significantly (P < 0.05) higher reducing power than the shoot.

Table 3. DPPH IC₅₀, Trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP) of the methanol extracts of the berry and shoot of *S. pseudocapsicum*.

Antioxidant activity	Berry	Shoot	Vitamin C
DPPH IC ₅₀ (mg/ mL)	2.33 ± 0.02 ^a	1.88 ± 0.01 ^b	0.04 ± 0.01 ^c
ABTS* scavenging ability (µM TEAC/g)	451.12 ± 0.67 ^a	422.88 ± 1.34 ^b	NA
FRAP(mg GAE/g)	8.74 ± 0.02 ^a	6.25 ± 0.07 ^b	NA

Data represent the mean ± standard deviation of triplicate readings. TE is Trolox equivalent. Values with different lowercase superscript letter along the same row are significantly different (P < 0.05). NA means "Not applicable".

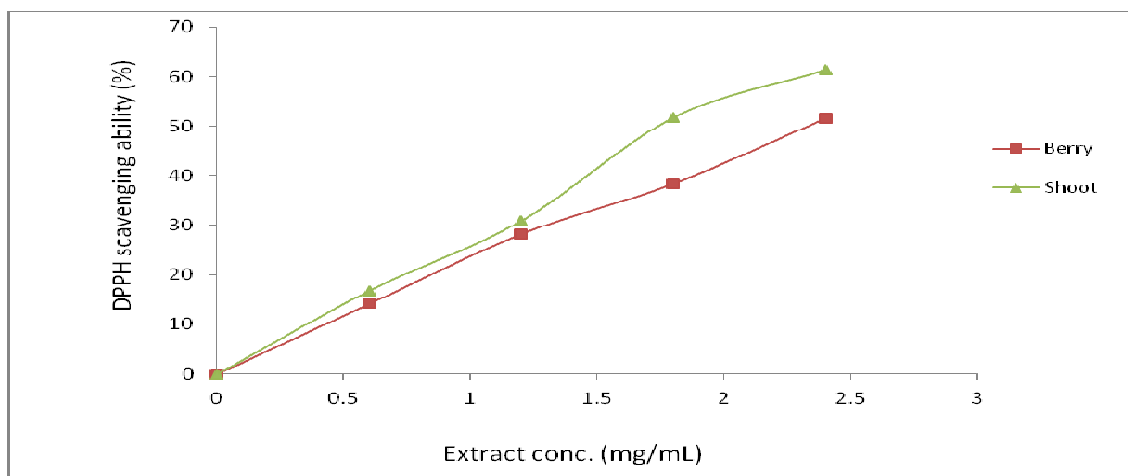


Figure 1. DPPH % scavenging ability-extract concentration curve of the methanol extracts of the berry and shoot of *S. pseudocapsicum*.

3. DISCUSSION

The distribution of the primary metabolites indicated that both the berry and the shoot of *S. pseudocapsicum* are good sources of protein. Proteins provide structural material for the human body; and they also function as enzymes, hormones, and antibodies. Dietary proteins are the major source of the essential amino acids for humans [34]. Plant storage proteins such as lectins are known to be involved in several biological processes, such as antimicrobial and antiviral action, and inhibitory effect on the growth of tumor cells [35]. Lipids, in addition to providing fuel for metabolism, are major components of cell membranes. Some plant lipids contain some bioactive polyunsaturated fatty acids (omega 3 and omega 6) that have been shown to be beneficial in preventing cardiovascular diseases, and decreasing the incorporation of cholesterol into the walls of arteries [36].

Intake of the berry and shoot of *S. pseudocapsicum* may not pose any glycemic problem, due to their low level of digestible carbohydrates (sugars and starches). Sugars and starches function primarily to provide energy to body cells, including the brain, which is entirely carbohydrate-dependent for its energy need. Sugars are involved in control of blood glucose and insulin metabolism, intestinal microflora activity, and food fermentation. Monosaccharides bound to protein and lipid molecules (glycoproteins and glycolipids) are involved in cell signaling [34]. The predominance of amylopectin over amylose in both the berry and the shoot of *S. pseudocapsicum* observed in this study is in agreement with the reports of Yotsawimonwat *et al.* [37] and Irondi *et al.* [38], who observed that amylopectin is the major component in most starch. Starch is composed of amylose (straight chains of glucose) and amylopectin (highly branched chains of glucose). Hydrolysis of amylose results in the release of fewer glucose molecules at once than the hydrolysis of the highly branched amylopectin chains. Thus, unlike amylopectin, high amylose content implies a lower glycemic index [39] due to its lower glucose response [40].

Vitamin C is a water-soluble antioxidant, acting as a leading natural antioxidant that can scavenge ROS, and it has anticarcinogenic effects [41, 42]. It promotes iron absorption, increases antibody concentration, and improves human immunity. In addition, vitamin C is beneficial in strengthening blood vessel and decreasing cholesterol concentration; it also helps to prevent arteriosclerosis-related cardiovascular diseases, as well as hypertension and apoplexy [43].

Phenolic compounds are considered as being a major group of plant secondary metabolites that contribute to the antioxidant activity of plants. In addition, they possess biological activities, such as anti-histamine [44], anti-inflammatory and anticarcinogenic [7], antibacterial [45], and antiviral activities [46]. Several recent studies have also reported that phenolic compounds present in extracts from different plants foods and their products, including soybean and *Mangifera indica*, inhibited carbohydrate-digesting gastric enzymes [47, 48], that have been implicated in the pathology of type 2 diabetes.

Saponins have a potential as pharmaceutical synthons, and they have been used in hormone synthesis [49]. They also have other biological activities including, hypoglycemic, antioxidant and hypolipidaemic activities; lowering of cancer risks, virucidal activity, reduction of protein digestibility and antimicrobial activity [50]. Alkaloids, as the most efficient therapeutically significant plant substances, have such pharmacological uses as anticancer agents, gout suppressant, muscle relaxant, antiarrhythmic, antibiotic, and sedative agents [51]. Sharma *et al.* [52] also reported that alkaloids are preventive or therapeutic agents against human free radical associated diseases.

The higher level of total carotenoid in the shoot than in the berry may be related to the presence of chlorophyll in shoot. Gitelson *et al.* [53] has earlier established a linear relationship between chlorophyll and carotenoids contents in higher plant leaves. Carotenoids are lipid-soluble antioxidant, which have been reported to have health benefits such as pro-vitamin A activity [54], antioxidant [55], anticancer [56], and antiobesity effects [57].

Increase in the free radicals and reactive oxygen species burden of the body cells, without a corresponding robust antioxidant defense system leads to oxidative stress, and this plays an important role in the pathogenesis of degenerative diseases [58]. Antioxidants react with DPPH, which is a stable free radical, and convert it to α,α -diphenyl- β -picryl hydrazine. As antioxidants donate protons to this radical, its absorption decreases. So the degree of discoloration indicates the scavenging potentials of the antioxidant extract [59]. The lower DPPH IC₅₀ of vitamin C, a reference antioxidant, in comparison with the berry and the shoot of *S. pseudocapsicum*, indicated that vitamin C was more potent in scavenging the DPPH free radical than the berry and the shoot. However, the scavenging ability of the shoot was stronger than that of the berry. Irondi *et al.* [60] noted that since IC₅₀ is a measure of inhibitory concentration, a lower IC₅₀ value indicated a greater antioxidant activity.

Extracts of both the berry and the shoot of *S. pseudocapsicum* also scavenged ABTS radical. The ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS* by antioxidants in a reaction that involves electron transfer process [7]. The ferric reducing antioxidant power (FRAP) of the extracts indicated their ability to reduce Fe³⁺ to Fe²⁺. In FRAP assay, potassium ferricyanide is used as an

oxidant, in a reaction that involves electron transfer, during which there is the reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to the coloured ferrous form [61].

4. CONCLUSION

The results of this study revealed that the berry and the shoot of *S. pseudocapsicum* contain appreciable levels primary and secondary metabolites, including protein, lipid, starch, sugar, amylose, amylopectin, vitamin C, total phenol, tannin, total flavonoid, total saponin, total alkaloid, and total carotenoid; and possess antioxidant activity *in vitro*. Comparatively, the berry might have more pharmacological benefits in alleviating oxidative stress in disease conditions than the shoot, due to its higher content of secondary metabolites and *in vitro* antioxidant activity.

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