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PHYTO-CONSTITUENTS AND ANTI-OXIDANT ACTIVITY OF THE PULP OF SNAKE TOMATO (*TRICHOSANTHES CUCUMERINA* L.)

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Abstract

The phyto-constituents and antioxidant activity of the fruit pulp of *Trichosanthes cucumerina* L. have not been reported in literature and were therefore studied. Two identified morphotypes of this plant (Morphotype I [V1] having long fruit with deep green background and white stripes; and Morphotype II [V2] having light green coloured long fruit) were used for the studies. The dry matter contents of the pulp of the V1 and V2 were 10.9 and 9.6 g/100g fresh weight (FW), while the ascorbic acid contents were 25.7 and 24.8 mg/100g fresh weight (FW), and lycopene contents were 18.0 and 16.1 mg/100g FW, respectively. The total phenolics, total flavonoids and total ferric reducing antioxidant power (FRAP) of V2 were significantly higher ($P < 0.05$) than that of V1 by 46.8%, 78.0% and 26.2%, respectively. Bulk of the carotenoids is made up of lutein in the concentration of 15.6 and 18.4 mg/100g FW, for V1 and V2, respectively. The α -carotene contents were 10.3 and 10.7 mg/100g FW while the β -carotene contents were 2.4 and 2.8 mg/100g FW for V1 and V2, respectively. It is concluded from the results of this study that the two morphotypes of *T. cucumerina* possess valuable nutraceutical properties that can qualify them as viable substitute to the Solanaceous tomato.

Key words: *Trichosanthes cucumerina* L., phenolics, flavonoids, antioxidant power

Introduction

The Food and Agricultural Organization of the United Nations (FAO)(1998) reported that countries of West and Central Africa sub-regions have a large number of under-utilized indigenous edible plant species that are important to the livelihoods of local population. Numerous scientific studies have shown the importance of indigenous edible plants in the nutrition of the rural human population in Africa (Chweya, 1996; Abukutsa-Onyago, 2003; Adebooye, 1996; Adebooye *et al.*, 2001). All over Africa, these traditional food plants have been major sources of nutrients for rural dwellers that cannot pay for milk, egg and milk (Adebooye, 1996). One of such high premium indigenous food plant is *Trichosanthes cucumerina* L. Adebooye *et al.* (2006) reported that *Trichosanthes cucumerina* L., a member of the Cucurbitaceae, also locally known as Snake Tomato, is utilized as a substitute to the Solanaceous tomato (*Lycopersicon esculentum* (L.) Mill.) due to its sweet tasting, aromatic, and deep red endocarp pulp when fully ripe and that the fruit pulp does not go sour as quickly as paste of *L. esculentum*. These good qualities have made this plant a substitute to the Solanaceous tomato especially during the off-season when prices of Solanaceous tomato are very high, suggesting that consumption maybe related to income level. The pulp is a good source of ascorbic acid (23.1-23.3 mg/100 g), which is far higher than that of the popular Solanaceous tomato varieties in Nigeria, thereby suggesting its possible use for paste and puree (Adebooye *et al.*, 2005). Fruits and vegetables are good sources of natural antioxidants for the human diet, containing many different antioxidant

components which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline in addition to other health benefits (Cao *et al.*, 1996; Wang *et al.*, 1996; Velioglu *et al.*, 1998; Cohen *et al.*, 2000; Liu *et al.*, 2000; Knekt *et al.*, 2002; Sweeney *et al.*, 2002; Amin *et al.*, 2004; Sahlin *et al.*, 2004). These positive effects are believed to be attributable to the antioxidants, particularly the carotenoids, flavonoids, lycopene, phenolics and β -carotene (Lavelli *et al.*, 2000; Amin *et al.*, 2004; Zhang and Hamauzu, 2004). Despite the good qualities of the fruit of *T. cucumerina* and its wide consumption by the poor rural population in Nigeria, research has not attempted studying the activity and components of antioxidant of this plant. This study was therefore designed to study the activity and components of antioxidant in two Nigerian indigenous morphotypes of *T. cucumerina* that had earlier been reported in literature (Adebooye *et al.*, 2005; Oloyede and Adebooye, 2005; Adebooye and Oloyede, 2006).

Materials and Methods

Plant material

Fully ripe fruit pulp of two morphotypes of *T. cucumerina* that were produced at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria were used for this study. Morphotype I [V1] have long fruit with deep green background and white stripes at unripe stage while Morphotype II [V2] have light green coloured long fruit when at unripe stage. The fruits were split open and the pulp was extracted. The pulp for each morphotype was freeze-dried and stored until required for analyses.

The following determinations were carried out:

Ascorbic acid determination

For ascorbic acid determination the method described by Sahlin *et al.* (2004) was used. Ascorbic acid was measured by titration with phenolindo-2, 6- dichlorophenol (DPIP). The powder (0.2 g) was mixed with 40mL of a buffer solution made up of 1 g/L oxalic acid and 4 g/L sodium acetate anhydrous. This was titrated against a solution containing 295 mg/L DPIP and 100 mg/L sodium bicarbonate. The results were expressed as mg/100 g FW and DM.

Total phenolics determination

Total phenolic was determined by employing Folin–Ciocalteu reaction, as determined by Zhi and Howard (2005). About 2 g sample was added to 25 mL double distilled water (pH 7.0) at 100 °C. The sample was homogenized with a dismembrator followed by the transfer of the homogenate. The homogenate was transferred to a rotary shaker (Remi Instruments, India) for 6 h to ensure full extraction. The homogenate was thereafter filtered, the filtrate was made up to 50 mL and then centrifuged at 3800 rpm for 10 min. The supernatant was used for total phenolics determination. Two hundred microliter of supernatant was added to 1 mL of 1:10 diluted Folin Ciocalteu reagent. After 4 min, 800 microliter of sodium carbonate (75 g L⁻¹) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0–100 mg L⁻¹) was used for calibration of a standard curve. The results were expressed as gallic acid equivalents (GAE) per 100 g FW and DM.

Total tannins determination

The method described by Pearson (1976), with slight modification, was used for the determination of tannin content of samples. Extraction of tannin was achieved by grinding 2 g of freeze-dried sample in 50 mL distilled water in pestle and mortar. The homogenate was transferred to a rotary shaker (Remi Instruments) for 12 h. Thereafter, the homogenate was filtered and the filtrate (extract) made up to 50 mL. One millilitre of the extract of standard tannic acid (0.01 g L⁻¹ tannic acid) was measured into different test tubes and 1 mL of Folin–Denis reagent was added to each test tube followed by 2.5 mL of saturated sodium carbonate solution. The solutions were made up to 10 mL mark with distilled water and shaken to mix properly. Thereafter, reaction mixtures were incubated at room temperature (approximately 30 °C) for 30 min. The absorbance was measured against the reagent blank (containing 1 mL distilled water in place of extract or standard tannic acid solution) in a UV–Visible spectrophotometer Model UV 1601 version 2.40 (Shimadzu) at 760 nm.

Total flavonoids determination

The aluminium chloride colorimetric assay was used for total flavonoids determination, as described by Marinova *et al.* (2005). Extraction of flavonoids was achieved by homogenizing 2 g of the pulp in 50 mL distilled water in pestle and mortar. The homogenate was transferred to a rotary shaker (Remi Instruments) for 12 h. The suspension was filtered and the filtrate (extract) made up to 50 mL. Precisely, 1 mL of extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg L⁻¹) was added to test tubes containing 4 mL of double-distilled water. To the mixture was added 0.3 mL 5% NaNO₂. After 5 min, 0.3 mL 10% (w/v) AlCl₃ was added, then 2 mL of 1M NaOH and the total volume was made up to 10 mL with double-distilled water. The solution was mixed thoroughly and the absorbance of both the samples, blank and standard, were read at 510 nm using UV-Visible spectrophotometer Model UV 1601 version 2.40 (Shimadzu). Total flavonoids content was expressed as mg catechin equivalents (CE per 100 g FW and DM).

Lycopene determination

Lycopene was extracted in the dark from 100mg of the sample powder using 5mL of heptane for 10 min at 45 °C, then for a further 30 min on a rotary mixer at 5–7 °C and then filtered (Sahlin *et al.*, 2004). Further 3mL heptane was added to the residues, mixed vigorously for 1 min and the supernatant was pooled with the first. The extraction was again repeated with 3mL of heptane. The absorbance of the combined heptane extract was measured at 470nm and the concentration of lycopene was calculated using molar molar extinction coefficient of 3450 M⁻¹cm⁻¹ (Van het Hof *et al.* 2000).

Carotenoids components determination

Carotenoids were analyzed according to the methods described by Zhang and Hamauzu (2004). Briefly, 15 g of powdered samples were homogenized with 10 ml acetone at -20 °C. The homogenate was filtered with four layers of cheesecloth. The residue was treated with acetone (-20 °C) for three successive extractions until the green colour could no longer be visually detected in the extract and residue. The filtrate was combined and centrifuged at 4000rpm for 10 min. The supernatant was collected and then filtered through a 0.45 µm Advantec filter pore for HPLC analysis. Samples were separated on a Luna 5µ C18 column (150 x 4.60 mm from Phenomenex) at 40 °C by an HPLC (SHIMADZU LC-VP Liquid Chromatograph equipped with a SPD M10Avp photodiode array detector). The mobile phase consisted of acetonitrile : water (9:1, solvent A) and ethyl acetate (solvent B). The flow rate was 1.0 ml/min. The following gradient elution was used for separation: 0% B at 0 min, 50% B at 20 min, and 50% B at 35 min. Samples were detected at 450 nm. Identification of carotenoids was tentatively achieved by their retention times and UV spectra, recorded with a SHIMADZU SPD-M10Avp photodiode array detector, in comparison with authentic β-carotene and carotenoids, identified according to Howard *et al.* (2000) and Mizda *et al.* (2002). The contents of carotenoids were expressed as milligramme per 100 g FW and DM.

Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant power (FRAP) assay was carried out in triplicate according to the method of Benzie and Strain (1996). The FRAP reagent was prepared by mixing acetate buffer pH 3.6, Solution X and 20 mM iron (III) chloride solution in proportions 10:1:1 (v/v/v), respectively. Solution X was prepared by mixing 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) and 40 mM HCl. Fresh FRAP reagent was used for the analyses and was warmed to 37 °C prior to use. Fifty microliter of sample (2 g) (extracted in 20% (v/v) acetone) were added to 1.5 mL of the FRAP reagent. The absorbance was measured at 593 nm after 4 min incubation. The standard curve was constructed using Iron (II) sulphate solution (100-200 µM) and the results were expressed as mmol Fe (II)/100g FW and DM.

Table 1: Dry matter, lycopene, ascorbic acid, total phenolics, tannins, total flavonoids and ferric reducing antioxidant power (FRAP) of two morphotypes of *T. cucumerina* L⁺

	Dry matter (mg/100g)	Lycopene (mg/100g)		Ascorbic acid (mg/100g)		Total phenolics (mg/100g)		Total tannins (mg/100g)		Total flavonoids (mg/100g)		FRAP (mmol Fe ²⁺ /100g)	
		FW	DM	FW	DM	FW	DM	FW	DM	FW	DM	FW	DM
V1 ⁺⁺	10.9	18.0	165.3	25.7	235.8	55.0	504.2	27.3	250.4	11.7	107.0	20.6	189.0
V2	9.6	16.1	167.7	24.8	258.3	71.9	749.0	25.0	260.6	18.3	190.7	26.0	270.4
Lsd 5%*	ns	ns	ns	ns	ns	11.3	54.2	ns	ns	4.2	60.4	4.5	61.0

Each value is a mean of triplicate analyses.

*ns: not significant at 5% level of probability.

⁺ FW and DM : Fresh weight and Dry matter, respectively.

⁺⁺ V1 and V2 : Morphotypes I and II, respectively.

Table 2: Components of carotenoids of two morphotypes of *T. cucumerina* L.⁺

	Lutein (mg/100g)		α -carotene (mg/100g)		β -carotene (mg/100g)	
	FW	DM	FW	DM	FW	DM
V1	15.6	143.1	10.3	94.5	2.4	22.0
V2	18.4	191.7	10.7	101.5	2.8	29.2
Lsd5%*	1.2	20.1	ns	ns	ns	ns

Each value is a mean of triplicate analyses. *ns: not significant at 5% level of probability.

⁺FW and DM : Fresh weight and Dry matter, respectively. ⁺⁺ V1 and V2 : Morphoty

Data analyses

Each analysis was replicated three times in a completely random design (CRD) according to the method of Steele and Torrie (1995). Means, where significantly different were separated using the least significant difference at 5% level of probability. All the chemical analyses results were presented on fresh weight (FW) and dry weight (DW) basis to allow for proper comparison with the works of earlier authors, which were either presented as FW or DW.

Results and Discussion

The dry matter contents of the pulp of Morphotype I (V1) and Morphotype II (V2) were 10.9 and 9.6 g/100g FW, while the ascorbic acid contents were 25.7 and 24.8 mg/100 g FW, respectively (Table 1). These values are consistent with those reported by Adebooye *et al.* (2005) and Adebooye and Oloyede (2006) for the same morphotypes of *T. cucumerina*. The dry matter values are higher when compared with the dry matter contents of 8.3 g/100 g FW and 5.3 g/100 g FW reported for Aranca tomatoes and Excell tomatoes, respectively by Sahlin *et al.* (2004). The values obtained for ascorbic acid in this study are higher than those reported for Solanaceous tomato by Davey *et al.* (2000) (16 mg/100g FW) and Sahlin *et al.* (2004) (9.6 mg/100g FW for Excell Tomatoes). The ascorbic acid values are however comparable with 25.5-23.7 mg/100g FW reported for tomatoes by Burlingame *et al.* (1993) and Sahlin *et al.* (2004). Statistical analyses showed that there was no significant difference in the lycopene and total tannins contents of the two snake tomato morphotypes. The lycopene contents of the two morphotypes of *T. cucumerina* (18.0 and 16.1 mg/100g FW) were within the range of values (5.2- 42.0 mg/100g FW) reported for field-grown Solanaceous tomatoes by various workers including Gomez *et al.* (2001) and Takeoka *et al.* (2001).

The lycopene contents for *T. cucumerina* were however higher than 2.6- 3.9 mg/100g FW reported for two Solanaceous tomato varieties by Sahlin *et al.* (2004). The total tannins contents of the two morphotypes of *T. cucumerina* were 27.3 and 25.0 mg/100g FW. However, the total phenolics, total flavonoids and total anti-oxidant power (Table 1) of the two *T. cucumerina* morphotypes differ significantly ($P < 0.05$). The total phenolics, total flavonoids and total (FRAP) anti-oxidant power of V2 were higher than that of V1 by 46.8%, 78.0% and 26.2%, respectively. These differences could be attributed to genotype. Previous studies by Adebooye *et al.* (2005), Adebooye and Oloyede (2005) and Oloyede and Adebooye (2006) have shown clear genotypic differences between the two morphotypes of *T. cucumerina*. The higher FRAP obtained for V2 in this study could be attributed to its higher contents of phenolics and flavonoids compared to V1. High concentration of phenolics and flavonoids have been shown to command high free radical scavenging activity in plant (Zhang and Hamauzu, 2004; Sahlin *et al.*, 2004). The values of phenolics for *T. cucumerina* are comparable to the values reported for tomato by Sahlin *et al.* (2004).

Literature survey revealed that, there is no report on the anti-oxidant composition and anti-oxidant power of *T. cucumerina* fruit pulp in literature. However, earlier study on another member of Cucurbitaceae (pumpkin) using the Trolox equivalent antioxidant capacity (TEAC) assay showed that this vegetable contains high level of carotenoids (Muller, 1997), especially less lipid-soluble carotenoids such as lutein, which had high antioxidant efficiency in the TEAC assay (Miller *et al.*, 1996). The FRAP of the two morphotypes of *T. cucumerina* reported in this paper (189.0 and 270.4 mM Fe^{2+} / 100g DM) are higher than those that were reported for different melons that are also members of Cucurbitaceous family by Buratti *et al.* (2000), Halvorsen *et al.* (2002), Leong and Shui (2002) and Nicoletta *et al.* (2003).

Table 2 shows that the carotenoids components of the two morphotypes of *T. cucumerina* did not differ significantly. Results also showed that bulk of the carotenoids is made up of lutein (143.1-191.7 mg/100g DM) and V2 had significantly higher lutein content than V1. The different values obtained for lutein, α -carotene and β -carotene in this study fall within the 0.06 to 7.4 mg/100g for α -carotene, 0 to 7.5 mg/100g for β -carotene and 0 to 17 mg/100g for lutein reported for pumpkin (Cucurbitaceae) by Murkovic *et al.* (2002). The high antioxidant power in *T. cucumerina* fruit pulp was suspected to be related to its high contents of lycopene, ascorbic acid, carotenoids, total flavonoids and phenolics because, earlier studies on fruits have shown that high antioxidant capacity is dependent on high content of phenolic acids, carotenoids, vitamin C and flavonoids such as anthocyanins (Halvorsen *et al.*, 2002; Kähkönen *et al.* 1999; Kalt *et al.*, 1999; Macheix *et al.*, 1999; Häkkinen *et al.*, 1999 and Gorinstein *et al.*, 2001).

It can be concluded from the results of this study that the two morphotypes of *T. cucumerina* possess valuable nutraceutical properties that man must take advantage of. The fruit pulp contains ascorbic acid, lycopene, phenolics, flavonoids and antioxidant power that are comparable to that of Solanaceous tomato and are higher than those of most widely eaten members of Cucurbitaceae.

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