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Effects of extraction solvent, morphological parts and ripening stage on antioxidative activity of *Solanum anguivi* fruit

Daramola, B.

Department of Food Technology Federal Polytechnic, PMB 5351, Ado-Ekiti, Ekiti State, Nigeria

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Abstract

Effects of extraction solvent namely ethanol, diethylether-chloroform mixture, morphological matrix namely fruit pericarp seed and three ripening stages namely green, yellow and red on antioxidative activity of *S. anguivi* fruit were studied. Phytochemical screening and assessment of some antioxidative markers were evaluated. Result showed that all samples were endowed with variable amounts of reductones, phenolics, alkaloids and saponins. Quantitative (mg ascorbic acid activity equivalent/ mg sample) assessment showed that antioxidative activity of ethanolic derived extracts were high in terms of total phenolic content (TPC) (75.00-196.88), relative reducing power (RRP) (165.00-889.29) and radical scavenging activity (RSA, %) (64.00-85.50) in comparison to low antioxidative activity in terms of TPC (2.81-45.00), RRP (18.21-25.71) and RSA (14.10-40.00) of diethyl ether derived extracts. With respect to morphological matrix, antioxidative activity was in the order : seed fruit pericarp. Stage of ripeness conferred no significant increase on antioxidative activity.

Introduction

Phytochemicals are group of plant inherent bioactive substances that are responsible for protection of such plants from environmental stress, microbial attack, insects and other external aggression. These phytochemicals are localized to fruit, seed, stem epidermis, flower and other peripheral surfaces of plants (Daramola and Adegoke, 2011). Also called secondary metabolites, phytochemicals include, flavonoids, alkaloids, saponins, terpenoids, anthraquinone and carotenoids (Trease and Evans, 2002). The therauptic values of these secondary metabolites have been harness in treatment and management of public health 'World-Over. One of the activities of this group of bioactive constituents is term antioxidant. The importance of antioxidant can be illustrated in the oxidation process phenomenon. Oxidation processes are inevitable in living system, however they are associated with production of free radicals. The free radicals are undesirable in food, drug and living system because they are linked with majority of human diseases notably, ageing, atherosclerosis, cancer, diabetics, liver cirrhosis, cardiovascular disorders etc. Also, free radical could result to spoilage of food leading to loss of nutritional and sensory quality (Gutteridge, 1995; Aruoma, 1998). The reactive oxygen species (ROS) can be in one or more of the following: super oxide anion OO., peroxy/ radical (ROO.) reactive hydroxyl (OH.), nitric oxide (NO.) and peroxy nitrite anion (NOOO.) Antioxidants are capable of ceasing direct ROS attacks and radical mediated oxidative reactions. Consequently, application of antioxidant prevents many diseases and health problems (Tepe and Sokmen, 2007). Also, antioxidants are important in extension of shelf-life of foods and drugs.

In terms of origin, antioxidant can be natural or synthetic. Although, synthetic antioxidants are effective in prevent oxidation but they are surrounded by league of limitations notably safety concern. However, natural antioxidants are effective and safe because they are parts of plants that man has been eating from prehistoric times. Natural antioxidants can be sourced from any part of plant; food fruit, seed, bark, flower, herb and other medicinal plants. Sourcing of antioxidants from natural means and application for treatment and management of public disease has been on the increase in recent times (Poumorad *et al.*, 2006).

Solanum anguivi Lam belong to the plant family Solanaceae and can be found as a wild plant in many places throughout the non arid parts of Africa notably Cote de Voire, Uganda and Nigeria (Elekofehinti *et al.*, 2012). The fruit is used in folklore medicine in treatment of high blood pressure (Schipper, 2000). The roots are useful in treatment of cough, ulcers, asthma, nervous disorder and fever (Zhu *et al.* (2000). Although ElekoFehinti *et al.* (2012) had worked on antioxidant activity of the whole fruit

*Corresponding author.
Email: *daramola_bode@yahoo.co.uk*

in relation to its saponin – antioxidant – enzymes. Also, Gandhippan and Rengasamy (2012) carry out antioxidant studies on leaves of some species of Solanacea family. However, none of the studies considers the effect of solvent-type, morphological matrix, fruit morphological matrix and level of ripeness. Consequently, the aims of this study were to study the influence of type of extraction-solvent in relation to three morphological parts (fruit, seed, pericarp) and level of ripeness on antioxidant activity of *S. anguivi* fruit.

Materials and Methods

Plant material

The fruits of *Solanum anguivi* Lam were purchased from commercial market in Ado-Ekiti, Nigeria. Taxonomic position was authenticated by the Agricultural Technology Dept, of Fed Polytechnic Ado-Ekiti. Nigeria.

Analytical chemicals

Diethylether, Chloroform Ethanol, 2,2,-diphenyl-l-picryl hydrazyl (DPPH), Trichloro acetic acid (TCA), potassium ferric cyanide, ferric chloride, Folin-ciocalteu reagent, sodium carbonate were analytical grade.

Preliminary processing

The fruits were separated into three classes based on colour then dried and manually separated into fruits, pericarp and seed.

Extraction procedure

Each sample was milled and subsequently extracted with ethanol at solid solvent ratio of 1:10 for 8hrs in a soxhlet apparatus. The refluxed samples were separated from the residues by filtering through Whatman No 1 filter paper. The filtrates were dried using vacuum evaporator (Adegoke and Gopalakrishna, 1998).

Phytochemicals evaluation of the crude extracts

Phytochemical investigations on *S. anguivi* extract samples were performed as described by Trease and Evans (2002); Harborne (1984). The ethanolic crude extracts (200 mg/mL) of the samples were subjected to various chemical tests in order to determine the secondary metabolites present by employing the use of various methods as follows:

Test for reducing sugars

A sample of 0.5 mL of each of the extracts, 2 mL of a mixture (1:1) of Fehling's solution 1 (A) and Fehling's solution II (B) were added and the mixture were boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars.

Test for flavonoids

A sample of 1 mL of each of the extracts and a few drops of 10 % ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus.

Test for amino acids

A sample of 0.5 mL of each of the extracts was treated with few drops of ninhydrin reagent, heated in water bath, a purple colour indicated the presence of amino acids.

Test for alkaloids

A sample of 0.5 mL of each of the extracts was to 0.2 mL of 36.5 % hydrochloric acid and 0.2 mL Dragendroff's reagent. Production of orange precipitate denoted the presence of alkaloids.

Test for reducing compounds

A sample of 1 mL of each of the extracts was added to a few drops of 10% ferric chloride and potassium hexaferrate (III) solution. A green or blue colour indicated reducing activity.

Test for steroids

Sample of 0.5 mL of each of the extracts were evaporated and dissolved in 2 ml chloroform. This was followed by the addition of 2 mL of concentrated sulphuric acid carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of steroids.

Test for saponins

Sample of 1 mL of each of the extracts was diluted with 2 mL of double distilled water. This was followed by the addition of few drops of olive oil. Formation of soluble emulsion on agitation of the mixture indicated the presence of saponin.

Evaluation of total phenolic content

Total phenolic content was evaluated according to the method described by Taga *et al.* (1984). Briefly: A 100 µL of Folin-Ciocalteau reagent (2N wrt acid Fluka Chemic AG-Ch-9470 BUCHS) was added to each sample (20 µL) and well mixed after addition of 1.58 mL of water. After 30 seconds, 300 µL of 2% sodium carbonate solution was added and the sample tubes were left at room temperature for 2 h. The absorbance (A) of the developed blue colour

Table 1. Phytochemical screening of *S. anguivi* fruit morphology parts in relation to extraction solvent and stage of ripeness

Sample No	Solvent System	Morphological Matrix	Sample code	Reducing compounds	Reducing sugars	Steroids	Alkaloids	Flavonoids	amino acids	Saponins
1	Ethanol	Pericarp	MGP	+++	+	+	-	-	+	+
2			PRP	++	++	-	-	++	+	+
3			FRP	++	-	+	+	+++	++	+
4		Seed	MGS	+++	-	+	+++	-	++	+++
5			FRS	++	+	+++	++++	-	++	+++
6			PRS	+++++	++	+++	++++	-	++	+++
7		Fruit	MGF	++	+	+++	+++	-	+	++
8			PRF	+++	++	++	+++	+	++	++
9			FRF	+++	++	++	+++	++	++	++
10	DDE – $CHCl_3$	Pericarp	MGP	+++++	+	+	+	-	+	++
11			PRP	+++++	-	++	+	-	+	++
12			FRP	+++++	+	+	-	++	+	++
13		Seed	MGS	+++++	+	+++	++++	-	++	++++
14			PRS	+++++	++	+++	++++	-	++	+++++
15			FRS	+++++	++	+++	++++	-	++	+++++
16		Fruit	MGF	+++++	+++	+++	++++	-	+++	++++
17			PRF	+++++	+++	+++	++++	-	++	+++++
18			FRF	++++	+++	+++	++++	++	+++	++++

PRP – Partially ripe pericarp; MGS – fruit Mature green seed; MGP – Fruit Mature green pericarp; FRS – Fully ripe seed; FRW – Fully Ripe whole; MGW – Mature green whole; FRP – Fully ripe pericarp; PRW – Partially ripe whole; PRS – Partially ripe seed; 1 – 9=DDE – $CHCl_3$ (50mL) + 30mL); 10 – 18 = ethanol (80mL); DDE – $CHCl_3$ =di-ethylether-chloroform system

was measured at 750 nm using Unicam Helios & UV/VIS/Spectrophotmeter. A plot of A750nm against corresponding concentration was used to calculate phenolic content (g/g ascorbic acid equivalent).

Determination of relative reducing power

Reducing power of each sample was determined in accordance with the method of Oyaizu (1986). Simply, each sample (1 mg/mL) in ethanol (2.5 mL) was mixed with sodium phosphate buffer (pH 6.6). the buffered sample was mixed with conditioning reagents (1%K3-Fe-CN6, 10% TCA, 0.1% FeCl3) centrifuged, diluted using distilled water and absorbance was measured at 700 nm. Higher absorbance indicates a higher reducing power. A plot of A750nm against corresponding concentration was used to calculate phenolic content (g/g ascorbic acid equivalent).

Total flavonoids content determination

The total flavonoids contents were determined by colorimetric method and expressed as mg quercetin equivalent per g of dry weight, using the method described by Chang *et al*. (2002). 0.5 mL of each the extracts (prepared from 1mg of crude extract dissolved in 1ml of methanol) were mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride ($AICl_3$) solution, 0.1 mL of (IM) sodium hydroxide solution (NaOH) and 2.8 mL of ddH2O. The resulting mixtures were well mixed and incubated for 30 minutes at obscurity. The absorbance of the reaction mixture was measured at 430 nm with a UV/visible spectrophotometer. A plot of A430 nm against corresponding concentration was used to calculate the total flavonoids content.

Measurement of radical-scavenging activity

Radical scavenging activity of samples on DPPH was estimated according to the method of Yamaguchi *et al*. (1998). An aliquot of samples (200 µL, 0.31-2.5 mg/mL), ascorbic acid (0.04-1.25 mg/mL) was mixed with the 100 mM Tris-HCl buffer (800 µL, pH 7.4) and then added to 1mL of 500 µm DPPH in ethanol (final concentration of 250 µm). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation.

Scavenging effect (%) = [1- (absorbance of sample at 517 nm/absorbance of control at 517 nm)] x100

Results and Discussion

Solanum anguivi *fruit description*

In addition to the authentication of the identity, the fruit of *S. anguivi* has the following description. The fruit is spherical in shape with a water displacement volume range of 0.3 mL to 0.45 mL, with a diameter range of 4 mm to 7 mm with average weight of 0.25 g to 0.38 g.

Phytochemical assessment

The result of phytochemical screening of three principal morphological parts namely, pericarp, seed and whole fruit in relation to stage ripeness, namely;

Table 2: Spectral Characteristics of *S. Anguivi* fruit extracts

Sample No	Solvent System	Morphological parts	Sample code	λ	A	λ	A	λ	A	λ	A	λ	A	λ	A
1	Ethanol	Pericarp	MGP	200	0.7	300	0.3	400	0.4	550	24.0	600	55.0	746	105.0
2			PRP	260	0.2	300	0.3	550	12.2	650	92.0	760	103.0	-	-
3			FRP	200	0	400	1.2	550	27.0	640	92.0	740	97.0	-	-
4		Seed	MGS	200	0.2	300	0.1	450	7.5	500	16.6	630	103.0	750	120.0
5			FRS	200	0.1	300	0.1	400	6.0	480	25.0	560	39.0	635	114.0
6			PRS	200	0.2	300	0.1	400	0.7	500	9.4	550	19.2	800	79.0
7		Fruit	MGW	300	0.0	400	0.3	500	7.8	550	28.0	640	81.0	740	112.0
8			PRW	300	0	400	1.7	500	2.9	550	33.0	640	103	750	110.0
9			FRW	300	0.0	400	2.4	500	3.4	630	111.0	700	111.0	-	-
10	DDE – $CHCl_3$	Pericarp	MGP	200	0.2	300	1.0	400	0.3	450	7.0	520	19.0	610	60.0
11			PRP	-	-	400	0.4	500	6.4	640	49.0	750	52.0	-	-
12			FRP	300	0.0	400	0.7	550	32.0	640	100	760	102.0	-	-
13		Seed	MGS	300	0.2	400	1.4	500	14.0	560	27.0	640	80.0	770	96.0
14			PRS	300	0.0	400	1.7	500	18.0	-	-	830	51.0	-	-
15			FRS	260	0.4	300	0.5	500	19.1	650	54.0	760	55.0	-	-
316		Fruit	MGW	200	0.0	470	10.6	560	24.0	640	70.0	720	95.0	-	-
17			PRW	-	-	420	0.2	500	0.43	569	17.0	600	50.0	700	53.0
18			FRW	200	0.5	400	1.2	550	29.0	630	90.0	740	94.0	-	-

PRP – Partially ripe pericarp; MGS – fruit Mature green seed; MGP – Fruit Mature green pericarp; FRS – Fully ripe seed; FRW – Fully Ripe whole; MGW – Mature green whole; FRP – Fully ripe pericarp; PRW – Partially ripe whole; PRS – Partially ripe seed; 1 – 9 – $CHCl_3$ + DDE (50 mL) + 30 mL); 10 – 18 = ethanol (80 mL); DDE – $CHCl_3$ – di-ethylether-chloroform system
λ = are the wavelength scanned with corresponding peak-max and associated absorbance(A)

green, half-ripe (yellow) and ripe (red) of *Solanum anguivi* extracted using two solvent systems; ethanol and mixture of diethyl ether: chloroform is shown in Table 1. The foremost observation was that the bioactive components were best extracted by ethanolic solvent in comparison to diethylether-choloroform mixture system. Irrespective of the stage of ripeness whole fruit samples were characterized by phenolics and reductones as screened using potassium hexacyano ferrate (iii) –iron (iii) chloride system. However, higher detection intensity was observed for ethanolic extract in comparison to diethylether-choloroform extract. Similarly results was earlier reported by Gandhiappan and Rengasamy (2012) on phytochemical endowment of *S. anguivi* methanolic extract, although level of ripeness was not taken into consideration by the authors in their studies.

In addition, all the six samples of the whole fruits of different ripe stages showed test positive for alkaloids and saponins. This is in agreement with an earlier report by Mandal and Ghosal (2012). However, other phytochemicals such as steroids, protein, and reducing sugars were detected at smaller strength in comparison to alkaloids and total phenolics. Flavonoids were detected only in ripe and half-ripe in whole and pericarp samples.

Considering, phytochemcial assessment with respect to morphological part (Table 1), it can be seen that the result of the phytochemical assessment of the whole fruit and seeds samples (Table 1) have similar phytochemcial test positivity strength. However, lesser phytochemical test positivity strength was observed for the pericarp-samples. Considering, the above discussion, it can be inferred that phytochemical-bioactive components of *S. anguivi* is majorly resident in the seed and not the pericarp. It is important to note that there is no significant mesocarp in the fruit of *S. anguivi*. Regarding ripeness level, presence of flavonoids was detected in ripe and half ripe pericarp and whole fruit. However, this did not translated to higher impact on total reductone marker and phenolic content.

Spectral characteristics of extract samples of S. anguivi

The spectral characteristics of *S. anguivi* extract samples are shown in Table 2. Spectral reading ranges between 200 nm and 700 nm (Silverstein *et al*., 1981). Absorption could be divided into 2; namely UV 200-400 nm and VIS-region 400-650.UV-absorption of extracts of diethylether- chloroform mixture system is lower comparison to the UV- absorption of ethanolic extracts in absolute terms. Similarly, by comparism, UV – absorption appeared to be pronounced in ripe and half-ripe pericarp or ripe whole fruit (pericarp + seed) than other samples. Absorption in the ultraviolet region by samples is a diagnostic feature of unsaturation or unbound electron in the absorbing molecules. (Shriner *et al*., 1979). Free election is a pre-requisite for antioxidative activity (Giese, 1996). However, absorption of seeds samples was low in comparison to other samples, yet showed high reductone content and total phenolics. This implies

Table 3. Selected antioxidant indices

Sample No	Solvent System	Morphological parts	Sample code	Total Phenolic Content	RRP	DPPH (%)	Total Flavonoids
1	DDE – $CHCl_3$	Pericarp	MGP	31.88	23.57	14.7	15.94
2			PRP	26.25	25.71	16.00	13.13
3			FRP	2.81	18.21	15.0	1.04
4		Seed	MGS	33.75	24.64	16.8	16.88
5			FRS	32.00	25.71	14.5	17.60
6			PRS	33.75	53.57	70.0	20.25
7		Fruit	MGW	45.00	26.79	15.0	25.65
8			PRW	24.38	22.50	25.0	13.41
9			FRW	30.00	26.79	14.1	15.90
10	Ethanol	Pericarp	MGP	136.88	375.00	84.0	50.81
11			PRP	90.00	619.29	84.9	63.00
12			FRP	75.00	246.43	87.2	50.00
13		Seed	MGS	196.88	889.29	66.5	108.28
14			PRS	103.13	187.50	82.3	61.88
15			FRS	78.75	165.00	86.3	47.25
16		Fruit	MGW	161.25	889.29	85.7	112.88
17			PRW	103.13	185.36	85.5	58.75
18			FRW	93.75	889.29	85.9	84.38

PRP – Partially ripe pericarp; MGS – fruit Mature green seed; MGP – Fruit Mature green pericarp; FRS – Fully ripe seed; FRW – Fully Ripe whole; MGW – Mature green whole; FRP – Fully ripe pericarp; PRW – Partially ripe whole; PRS – Partially ripe seed; 1 – 9 – $CHCl_3$ + DDE (50mL) + 30mL); 10 – 18 = ethanol (80mL); DDE – $CHCl_3$ – di-ethylether-chloroform system
TPC = mg ascorbic acid activity equivalent/ g sample , RP = mg ascorbic acid activity equivalent/g sample
DPPH = % inhibition (Scavenging effect)

or signalled the absence of low simple phenolics and other aliphatic hydroxyl compounds. Considering the vis-region, absorption increase in all samples as wavelength increased with higher absorption in ethanolic extract samples.

Quantitative assessment of some biochemical and antioxidative indices

The result of effect of solvent type, fruit morphological part and level of ripeness on quantitative antioxidative potentials of S. anguivi is shown in Table 3.

Total phenolic content

The total phenolic contents (mg ascorbic acid activity equivalent/mg sample) of diethylether-chloroform mixture extract samples were low (2.81-45.00) in comparison to the high (75.00 – 196.88) total phenolic content of ethanolic extract samples. The total phenolic content of the seeds (32.00-33.75; 78.75-196.88) and whole fruit (24.38-45.00; 93.75-161.25) are similar to each other but high in comparison to the low value of total phenolic content of the pericarp (2.81-31.88); 75.00-136.00 for diethylether-chloroform mixture and ethanolic solvent extracts respectively. From these results, it is clear that the total phenolic constituents of *S. anguivi* are majorly localized in the seed.

Relative reducing power

The trend of the result of the relative reducing power (mg ascorbic acid reducing activity equivalent) is similar to the result of Total phenolic content. The relative reducing power (mg ascorbic acid activity equivalent/mg sample) of the diethylether-chloroform mixture extract samples were low (18.21-53.57) in comparison to high (165.00-889.29) relative reducing power of ethanolic – extract samples. The relative reducing power of the seed (24.64-53.57; 165.00-889.29), whole fruit (22.50-26.79; 185.36-889.29) were high in comparison to low (18.21-25.71; 246.43-619.29) relative reducing power of extracts of pericarp.

Radical scavenging activity on DPPH

The radical scavenging activity (RSA) (%) of the diethylether-chloroform mixture extract samples range from 14.10-40.00. while the RSA (%) of ethanolic extract ranged from 64.00-85.50, examination of the RSA result in the Table 3 showed that RSA of seed fruit extract were higher in comparison to RSA (%) of the pericarp.

Total flavonoids

The total flavonoids (unit) of the diethylether-chloroform mixture extract is low (1.04-20.25) in comparison to the total flavonoids of the ethanolic extract samples. Similar to previous results, the total flavonoids in the seed and fruit were high in comparison to low total flavonoids in the pericarp.

Table 4. Antioxidantive activities of selected *S. anguivi*

Antioxidative Reaction	Antioxidant Sample	K (mL/mg)	EC_{50} (mg/mL)
RSA	PRW12	3.466	0.1998
	PRP	2.335	0.2968
	MGS	4.062	0.1706
RRP	PRW	3.172	0.2185
	PRP	2.695	0.2572
	MGS	4.500	0.1540

PRW= Partially ripe whole, PRP= Partially ripe pericarp , MGS= fruit Mature green seed

Concentration dependent kinetics for antioxidative activity

Antioxidative effects exhibited by the *S. anguivi* selected ethanolic extracts depended strongly on its concentration. In general, the antioxidative activity increased with antioxidant concentrations to a certain extent, then levelled off with further increase in the concentrations. The antioxidative activity of the extract of *S. anguivi* can be expressed by the following equation (Lai *et al*., 2001)

$$1 - A/A^* = e^{-kc}$$

Where A is the antioxidative activity at any given antioxidant concentration, A^* is the equilibrium antioxidative activity that remains constant over a large antioxidant concentration, C is the antioxidant concentration and K is a proportional constant. The proportional constant (K) can be obtained from the slope of a ln $(1-A/A^*)$ against antioxidant concentration (C) plot. Therefore, the proportional constant (K) has the unit of reciprocal concentration indicating the rate or degree of antioxidant activity. In addition, for every antioxidant reaction, the half-inhibition concentration (EC_{50}) which means the antioxidant concentration required for providing 50% of the antioxidative activity can be calculated Chou *et al.* (2002).

Antioxidant activity constant, EC_{50}

Three samples from ethanolic extract samples of Solanum anguivi fruit been representation of each of the morphological matrix were selected for determination of antioxidant activity constant and EC_{50}. Table 4 showed the summary of antioxidant activity constant and EC_{50} of the three classes of morphological part, namely the fruit, seed and the pericarp for DPPH and RRP assessments. From the table (Table 4), the order of antioxidant activity constant is MGS > PRW> PRP for both DPPH and RRP. Similarly, the result of EC_{50} showed in a reverse order that lesser amount of the sample were required to accomplished EC_{50} activity in the following order : MGS < PRW < PRP.

Conclusion

The activity/potency of antioxidative bioactive constituents of *S. anguivi* extracted using ethanol is superior to the antioxidative activity bioactive constituents extracted using DDE- $CHCl_3$ as revealed by phytochemical screen test and all the quantitative antioxidant marker examined in this study. This study indicated that the antioxidative activity of the seed and the fruit were similar but high in comparison to the low antioxidant activity of the pericarp. The technological implication of this study is that the results dictate the best solvent to extract active components of *S. anguivi*; Also the seed should be chosen for colourless antioxidant. While the fruit should be allowed to ripe and the pericarp could be used for production of yellow or red product which would be rich in carotenoids that could serve as dietary colourant.

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