PHYTOCHEMICAL CONSTITUENTS AND *In Vitro* ANTIOXIDANT ACTIVITY OF VARIOUS SOLVENT FRACTIONS OF *Solanum Melongena* L. (ROUND AND OVAL VARIETIES) DURING RIPENING

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ABSTRACT

Solanum melongena (round and oval varieties) are eggplants that are commonly consumed. The phytochemical constituents and *in vitro* antioxidant activity of these eggplants were determined during ripening. The *in vitro* antioxidant activity of the aqueous, aqueous-methanol, methanol, and acetone fractions of these eggplants was determined by the DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging assay and compared with ascorbic acid (vitamin C), a standard antioxidant. DPPH radical scavenging activity values of the aqueous-methanol, methanol, and acetone fractions of the eggplants were higher than that of ascorbic acid (23.28±0.34% for an ascorbic acid concentration of 0.001g/ml) at all stages of ripening. Analysis of the quantitative phytochemical constituents of *Solanum melongena* (round and oval varieties) revealed the presence of tannins, phenols, saponins, flavonoids and alkaloids in the fruits at all the stages of ripening. Flavonoids concentration increased continuously in the fruit of the round variety during ripening, while saponins and flavonoids concentration increased continuously in the fruit of the oval variety. The amount of flavonoids in the round variety (3.07± 0.09% dry weight) was however twice the amount recorded for the oval variety (1.50± 0.06 % dry weight). Increase in the levels of saponins and flavonoids have been correlated with high antioxidant activity. Aqueous-methanol, methanol, and acetone were the most effective extraction solvents for the estimation of antioxidant activity in both varieties of *Solanum melongena*. The study has shown that *Solanum melongena* (round and oval varieties) are excellent sources of phytochemicals. The concentration of phytochemicals such as flavonoids and saponins were higher in overripe fruits, therefore, the consumption of these fruits especially in the overripe stage should be encouraged.

INTRODUCTION

Eggplants also known as *Solanum* species are economic flowering plants belonging to the family *Solanaceae.* They are found throughout the temperate, sub-tropical and tropical regions of the world (Chen *et al*., 2001), however they are grown extensively in the sub-tropical and tropical regions (Ubokudom *et al.,* 2010). Eggplants comprise of diverse species, with great variation in morphology (Mueller, 2005; Sekara, 2007). The fruit exists in a wide array of shapes, sizes and colours (Chen *et al*., 2001). The name eggplant was derived from the shapes of the fruits of some varieties, which are white and shaped like chicken eggs (Akanitapichat *et al.,* 2010). Eggplants are consumed on daily basis by urban families and also represent the main source of income for some households in West Africa (Danquah- Jones, 2000).

The fruits of Eggplants are low in calories and are rich sources of potassium, magnesium, calcium and iron which are beneficial to human health. Eggplants are also used in the treatment of diabetes, asthma and arthritis. Extracts obtained from eggplants have helped in the reduction of blood and liver cholesterol in humans (Silva *et al.,* 2009). Nasunin a major component of the

anthocyanin of eggplant has been shown to inhibit lipid peroxidation (Igarashi *et al.,* 2003). Nasunin prevents cellular damage that can promote cancer and it also reduces free radical damage in joints which is a primary factor in rheumatoid arthritis (Bazzano *et al.,* 2002).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that can provide health benefits for humans (Eleazu *et al.,* 2012). They function as antioxidants and antimicrobial agents (Adesuyi *et al.,* 2012). Phytochemicals are present in the leaves, vegetables, roots and stems of plants (Akenga *et al.,* 2005).There are several classes of phytochemicals and these include alkaloids, flavonoids, saponins,tannins and phenols.

Antioxidants are the key species that retard the process of oxidative stress by reducing the level of reactive oxygen species, thus preventing a large number of chronic diseases (Seifried *et al.,* 2007). They play an important role in health maintenance and prevention of chronic and degenerative diseases such as atherosclerosis, carcinogenesis and neurodegenerative disorders (Uddin *et al.,* 2008) .Antioxidants prevent diseases by protecting cells against damaging effect of reactive oxygen species that cause oxidative stress, which results in cellular damage (Uddin *et al.,* 2008). Antioxidants are classified into enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase and glucose-6-phosphate dehydrogenase. Non-enzymatic antioxidants include minerals such as selenium, vitamins A, C and E, carotenoids (β-carotene, lutein, lycopene), organosulfur compounds (allium) and phytochemicals such as polyphenols, flavonoids and saponins (Prithviraj *et al.,* 2009). Food sources of antioxidants include fruits, vegetables, red wine, whole grains, seeds and nuts (Kohen and Nyska , 2011).

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals or reactive oxygen species in the human body (Abdalla and Roozen, 2001). The aim of this study was to investigate the effect of ripening on the antioxidant activity and quantitative phytochemical constituents of two varieties of *Solanum melongena* namely round and oval varieties.

MATERIALS AND METHODS

Plant materials

Fresh and unripe *Solanum melongena* fruits (round and oval varieties) were bought from Uselu market in Benin City, Nigeria. The fruits were left in the ripening chamber to ripen naturally. The unripe, ripe and overripe fruits (Figs.1a – 3b) were used for the analyses of their antioxidant activity and phytochemical constituents.

Fig. 1a: Unripe round *Solanum melongena*

Fig. 1b: Unripe oval *Solanum melongena*

Fig. 2a: Ripe round *Solanum melongena*

Fig. 2b: Ripe oval *Solanum melongena*

Fig. 3a: Overripe round *Solanum melongena*

Fig. 3b: Overripe oval *Solanum melongena*

Preparation of eggplant extract

The extraction was carried out by the method of Sulaiman *et al* (2011); The eggplants were washed, diced and ground into slurry by using mortar and pestle. Ten grammes (10 g) each of the eggplant slurry was separately extracted with 100 ml aqueous, aqueous-methanol, methanol and acetone in conical flasks. The mixture of eggplant slurry and the extraction solvent was stirred using a magnetic stirrer for 30 min at 1000 rpm. The extract obtained was filtered using a clean cheese cloth and centrifuged at 3000 rpm for 15 min. The supernatant was collected and used for the determination of antioxidant activity.

Determination of antioxidant activity

The antioxidant activity of the eggplant extract was determined by the DPPH free radical scavenging method of Brand-Williams *et al*. (1995) with slight modification. Precisely 2.5 ml of the eggplant extract was added to 7.5ml of 0.3 mM DPPH in methanol and incubated at 37^{o}C for 30 minutes. In the blank, 2.5 ml of distilled water was used to replace the eggplant extract. Absorbance was measured at 515 nm with a spectrophotometer. The percentage DPPH scavenging activity was determined as follows:

$$\% \text{ DPPH scavenging activity} = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Abs blank}} \times 100\%$$

The assay was carried out in triplicate. The percentage free radical scavenging activity of the eggplant extract was compared with that of ascorbic acid, a standard antioxidant.

Quantitative determination of the phytochemical constituents of *Solanum melongen*a fruits (round and oval varieties)

Alkaloid

Alkaloid was determined based on the Harborne method (1973). Five grammes of the sample were weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was

allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid

The flavonoid content of the extract was determined according to the method of Bohm and Kocipai-Abyazan (1994). 10g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. Aluminum chloride colorimetric method of Chang *et al.* 2002 was used to determine the flavonoids.

Saponin

The method used was that of Obadoni and Ochuko (2001). In this method, 100 ml of 20% aqueous ethanol was added to 20 g of the sample in a conical flask and heated over a hot bath for 4 hr with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight.

Tannin

Tannins were determined based on the method of Van-Burden and Robinson method (1981). Precisely 50 ml of distilled water was added to 5 g of the sample and shaken for 1 hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

Phenol

Total phenol was determined by spectrophotometric method. The sample was boiled with 50 ml of ether for 15 min for the extraction of the phenols. 5ml of the extract was pipette into 50 ml volumetric flask, 10ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. The absorbance was measured at 505 nm.

Statistical analysis

Statistical analysis was carried out using the SPSS computer package. Multiple comparisons were done using one way Analysis of Variance (ANOVA).

RESULTS

Tables 1 and 2 show the antioxidant activity of the round and oval varieties of *Solanum melongena* respectively as determined by the DPPH free radical scavenging ability of their unripe, ripe and overripe fruits, using different extraction solvents. The fruits exhibited potent free radical scavenging abilitythat increased with ripening.

Table 1: *Antioxidant activity of round *Solanum melongena* fruits at various ripening stages

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	**10.45±0.16^{a}	13.57±0.07^{b}	12.87±0.29^{b}
Aqueous-Methanol	25.14±0.17^{a}	30.01±0.06^{b}	35.13±0.07^{c}
Methanol	32.23±0.07^{a}	33.30±0.00^{a}	35.83±0.07^{b}
Acetone	33.33±0.00^{a}	33.21±0.04^{a}	34.43±0.07^{b}

**Mean ± SEM (n=3)
Means in the same row followed by different letters are significantly different at p<0.01
Means in the same row followed by the same letter are not significantly different at p<0.01
*Antioxidant activity was determined by the % DPPH scavenging activity of the fruits
Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.28±0.34

Table 2: *Antioxidant activity of oval *Solanum melongena* fruits at various ripening stages

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	**5.22±0.32^{a}	11.04±0.04^{b}	20.03±0.07^{c}
Aqueous-Methanol	25.80±0.00^{a}	27.36±0.07^{b}	29.14±0.07^{c}
Methanol	31.43±0.04^{a}	33.83±0.07^{b}	34.14±0.07^{b}
Acetone	33.92±0.07^{a}	33.04±0.00^{b}	34.65±0.04^{c}

**Mean ± SEM (n=3)
Means in the same row followed by different letters are significantly different at p<0.01
Means in the same row followed by the same letter are not significantly different at p<0.01
*Antioxidant activity was determined by the % DPPH scavenging activity of the fruits
Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.28±0.34

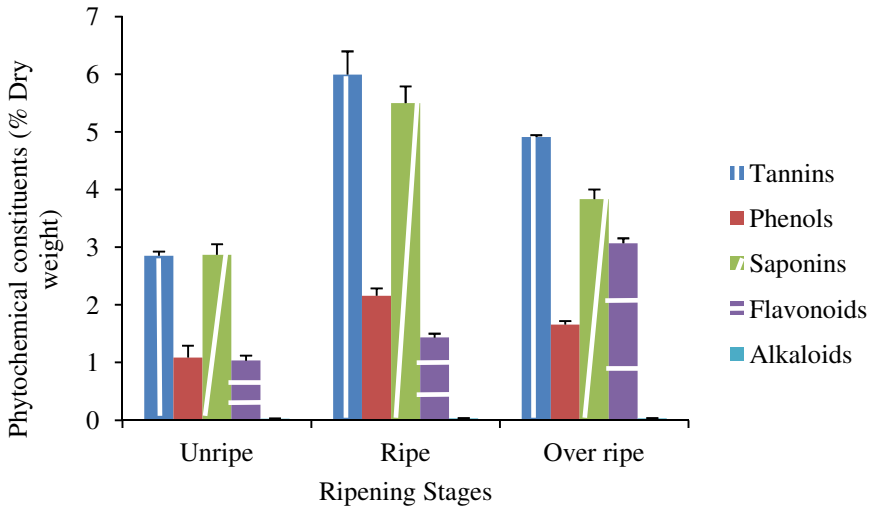

Fig. 4a:Phytochemical constituents of round *Solanum melongena* fruits at various ripening stages

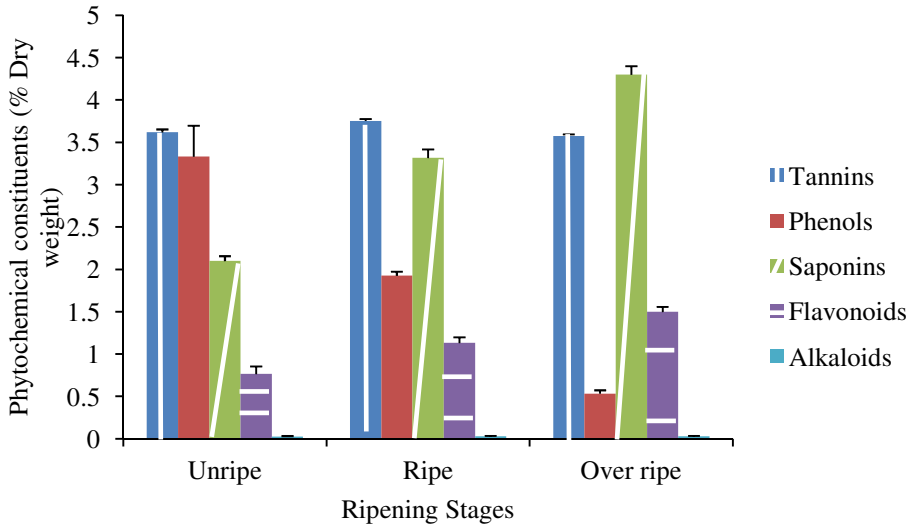

Fig 4b: Phytochemical constituents of oval *Solanum melongena* fruits at various ripening stages

Analysis of the phytochemical constituents of the fruits of both varieties of *Solanum melongena* revealed the presence of tannins, phenols, saponins, flavonoids and alkaloids in all the ripening stages. The overripe stage of the round variety of the fruit of *Solanum melongena* had the highest level of flavonoids, while that of the oval variety had the highest levels of flavonoids and saponins (Figs 4a and b).

DISCUSSION

Fruit ripening is a complex process that involves biochemical and physiological changes that affect the colour, flavor, texture and constituents of fruits [Asthma and Abaci, 2013]. In this study, the antioxidant activity and phytochemical constituents of *Solanum melongena* fruits (round and oval varieties) were determined at various stages of ripening (Figs. 1a-3b), in order to assess how ripening affects the antioxidation components of the fruits.

The DPPH scavenging activity of aqueous extracts of both varieties of *Solanum melongena* fruits showed a significant increase ($p<0.01$) from the unripe (10.45±0.16%) to the overripe (12.87±0.29%) stage in the round variety and from 5.22±0.32 to 20.03±0.07% respectively in the oval variety (Tables 1 and 2). This correlates with the findings of Zuhair *et al*., (2013) who worked on papaya and observed that antioxidant capacity increased with ripening using aqueous extraction medium. In this study, the antioxidant activity obtained for the various stages of ripening of both varieties of the fruit by using aqueous extraction, was however lower than that of ascorbic acid (23.28±0.34%) the standard antioxidant.The antioxidant activity of both varieties of *Solanum melongena*, when extracted with aqueous - methanol solvent showed an increase from 25.14±0.19% in the unripe to 35.73±0.07% in the overripe for round variety, and from 25.80±0.04% in the unripe to 29.14±0.07% in the overripe for oval variety. These values were higher than that of the standard antioxidant. Addition of methanol to the aqueous medium increased the solubility of the antioxidant compounds that were present in the fruits and hence their antioxidant activity.

The better extracting power of aqueous –methanol solvent when compared to aqueous solvent indicates that the mixing of non-polar solvent with water increases the polarity index of solvents thereby enhancing the extraction power and this corresponds with the findings by Zuhair *et al.,* (2013) and Musa *et al.,*(2011) who found out that the polarity of a solvent enhances the solubility of antioxidant compounds.Thus, the polarity of solvents has a direct function in the extraction process, because it can raise the solubility of antioxidant compounds (Alothman *et al*., 2009). Therefore screening of extraction solvents for the determination of

antioxidant activity is very vital in obtaining a reliable result and accurate information about a fruit or plant. The DPPH scavenging activity of the methanol and acetone extracts was also higher than that of aqueous and the standard antioxidant. It significantly varied ($p< 0.01$) between the ripening stages with the overripe stage having the highest DPPH scavenging activity (Tables 1 and 2). This correlates with the report of Jacob and Shenbagaraman, (2011) on aqueous and acetone extracts of *Clerodedrum serratum* a seasonal leafy vegetable in which they observed that acetone has a higher extraction activity compared to the aqueous and other organic solvents. Our findings however suggest that aqueous-methanol, methanol and acetone are effective solvents for the extraction and determination of antioxidants activity in round and oval varieties of *Solanum melongena* fruits.

Tannins were detected in both varieties of *Solanum melongena* fruits but their concentrations were highest in the ripe stage. Haruenkit *et al.,* (2010) reported that tannins level in *Mon Thong durian* decreased with ripening. In both varieties of *Solanum melongena* fruits, phenols decreased with ripening. Similar results have been reported for *Citrus sinensis* in which the phenol content decreased with ripening (Rekha *et al.,* 2012). Polymerisation, oxidation and conjugation of bound phenolics during maturation or ripening affect phenolic composition (Gruz *et al*., 2011). In the oval variety, saponin increased with ripening with the overripe stage having the highest value. This is in agreement with previous report by Soumaya and Nair, (2014) on *Averrhoa bilimbi* in which the saponin level increased with ripening. Saponins are known to have strong antioxidant, antiviral and antifungal activities (George *et al.,* 2002). For both varieties, flavonoids increased with ripening with the overripe stage having the highest flavonoid value. Moreover the round variety that exhibited a decrease in the level of saponins in the overripe stage, had twice the amount (3.07 ± 0.09 % dry weight) of the flavonoids level recorded for the oval variety (1.50 ± 0.06 % dry weight). Previous findings on *Solanum anguivi* Lam berries (Gnagnan) showed an increase in flavonoids during ripening. The increase in the level of flavonoids in this fruit was correlated with the increase in its antioxidant activity (Dan *et al*., 2014). The alkaloids content in both varieties of *Solanum melongena* (round and oval) showed no significant increase from the unripe stage to the overripe stage (Figs 4a and b).

CONCLUSION AND RECOMMENDATION

The presence of appreciable amounts of phytochemicals and high antioxidant activity in aqueous-methanol, methanol and acetone fractions, which was higher than that of the standard antioxidant (ascorbic acid) in each variety of *Solanum melongena* is consistent with the idea of ranking *Solanum melongena* in the top ten high antioxidant containing fruits. The antioxidant activity obtained was also dependent on the solvent used. Aqueous-methanol, methanol and acetone presented the highest antioxidant activity when compared to aqueous. Screening of solvents in order to determine the most effective solvent for antioxidant activity estimation is very important.The antioxidant properties of *Solanum melongena* are closely linked to its phytochemical content and this study has unraveled the effect of ripening on its phytochemical constituents and antioxidant properties which could serve as an impetus in the global fight against degenerative diseases whose etiology has been linked to oxidative stress.The study has also shown that *Solanum melongena* (round and oval varieties) are excellent sources of phytochemicals. The consumption of these fruits especially in the overripe stage should be encouraged.

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