IN-VITRO CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF POLYPHENOLS EXTRACT OF RIPE GARDEN EGG (*Solanum gilo*)

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*Solanum gilo*
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**ABSTRACT**

This study sought to investigate and compare the HPLC/DAD characterization, nutrient composition of ripe and unripe garden egg fruits to see its potentials for use in animal feed and in industrial processes. Analyses were carried out on *Solanum gilo* ripe (SGR) and *Solanum gilo* unripe (SGU) dried samples. Proximate analysis and minerals such as (Ca, Na, K, Mg, Zn) as well as some anti-nutritional factors (phytates, tannins) were carried out on the powdered samples. Polyphenols content were identified and quantified using HPLC-DAD. Total phenols, total flavonoids and vitamin C content was determined, while its antiradical assay was examined as typified 1,1-diphenyl-2-picrylhydrazyl [DPPH] and 2,2-Azinobis (3-ethylbenzo-thiazoline-6-sulfonate [ABTS]. Results of the study revealed an increased in fat and crude fibre content while a slight reduction was reported in protein content but no significant changes was reported in the total carbohydrate content. HPLC-DAD analysis showed that gallic acid was the most abundant phenolics present. There was increased in some of the flavonoids due to ripening such as Ellagic acid, Isoquercitrin, Quercetrin and Kaempferol. The total phenols, flavonoids increased in SGR, there was no change in DPPH scavenging ability while ABTS scavenging ability increased with the SGR. It can be concluded that ripe garden eggs which is usually discarded could be of use as an excellent livestock feed source/additives.
1 Introduction

Globally, one-third of the edible parts of food produced for human consumption gets lost or wasted, about 1.3 billion tonnes per year (Elisabeth, 2014). Food is wasted throughout the food supply chains, from initial agricultural production down to final household consumption. In medium and high-income countries food is to a great extent wasted even if it is still suitable for human consumption (FAO, 2011).

In sub-Saharan Africa, over 50% losses in agricultural production dominate total losses throughout the food supply chains for fruits and vegetables (FAO, 2011). Losses during postharvest and distribution stages are also severe, which can be explained by deterioration of perishable crops in the warm and humid climate of many developing countries (Kasso & Bekele, 2016). Not only the losses of food but they also represent a similar waste of human effort, farm inputs, livelihoods, investments and scarce resources such as water (FAO, 2011). Studies have also suggested that wastage from agricultural production make up 30–60 % which can be use for human consumption and animal feed (Salemeedeb et al., 2017).

Recently, people becoming health conscious and consume large quantities of fruits and vegetables leading to the accumulation of more waste. Climacteric fruits are those that can be harvested when mature but before ripening has begun, these include banana, melon, papaya, and tomato and even some of the species of garden egg. The disposal of these ripe or overripe fruits and vegetables as waste is a serious problem and their disposal likewise poses health hazard to man and animals (Ezejiofor et al., 2011).

Waste minimization is the emerging face of waste management. It has been discovered that waste particularly those of considerable calorific value can serve as valuable raw material for the production of value added products such as industrial products or part of feed ingredients for livestock feed. This is indeed a big plus both for the various industries and public health programme of environmental health and safety (Ezejiofor et al., 2014). It was found that the waste from Solanum melongena skin has been used as colorant (Galio et al., 2014) and also used in the production of methane (Gunaseelan., 2004).

Solanum gilo “Garden egg” is an edible vegetable crop belonging to the family Solanaceae. It is the most important of the vegetable families which are essentially tropical in origin (Nwanna et al., 2013). Most species are wild but some of them such as S. aethiopicum, S. macrocarpon, S. kamba and S.gilo bear edible fruits in West African origin (Nwanna et al., 2016). Its fruits may be consumed freshly raw, dry or cooked. It is one of the most important vegetable crops in West Africa mostly in Nigeria as it is consumed daily and remains a source of income for many rural dwellers (Chioma et al., 2011; Nwanna et al., 2013). However ripening of S. gilo during post harvest makes them unsuitable for human consumption and often discarded as a waste. Therefore, in order to determine whether the ripe garden egg considered as waste products could be incorporated into livestock feed or not, present study have been undertaken to compare the nutrient composition of unripe and ripe fruit of S. gilo to assess its antioxidant properties ,characterize and quantify the polyphenol constituents content using High performance liquid chromatography.

2 Materials and Methods

2.1 Sample collection

Mature unripe and ripe garden eggs (S. gilo) samples were collected from a farm settlement in Akure, Ondo State, Nigeria. The identification of the samples was carried out at the Crop, Soil, and Pest management (CSP) Department of the Federal University of Technology, Akure, Nigeria.

Figure 1 Pictures of Solanum gilo ripe and unripe.
2.2 Experimental requirement

All the chemicals used were of analytical grade. Optical absorbance was measured with an UV-VIS spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

2.3 Sample Preparation

The 200g of garden egg samples (Ripe and unripe) were sliced into pieces, oven dried at 50°C using (Uniscope SM90 England) oven and milled into powder using a Warring heavy duty blender (Warring Products Division, New Hartford, Connecticut, USA). Subsequently, the powdered samples were subjected to proximate and mineral analyses as well as determination of anti-nutrient composition, antioxidant properties and HPLC-DAD analysis.

2.4 Determination of Nutrient and anti-nutrient

The nutrient compositions (ash, fat, carbohydrates, moisture content and crude fiber) of the garden egg samples were determined by using the standard method given by AOAC (1990) and the protein content was determined using the micro-Kjeldahl method. The phytate content was determined by the method of (Wheeler & Ferrel, 1971), based on the ability of standard ferric chloride to precipitate phytate in diluted HCl extracts of the samples. The tannin content was analyzed according to Makkar et al. (1993).

Furthermore, minerals content such as Ca, Cu, Mg, Fe and Zn were determined using the method of Makkar et al. (1993), using aliquots of the solutions of the while Na and K were determined using flame photometry Perkin-Elmer (1982).

2.5 Preparation of aqueous extract

The aqueous extracts of the garden egg samples were prepared by adding 2 g of each milled samples in 100 ml distilled water for 8 h in a shaker. Thereafter, the mixtures were filtered using filter paper; filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was used for the determination of total phenol content and crude fiber) of the garden egg samples were analyzed according to Makkar et al. (1993).

2.6 Determination of total phenols

The total phenol content of the powdered sample was determined by adding 0.5 ml of the sample extract to an equal volume of water and 2.5 ml 10% Folin-Ciocalteau reagent (v/v) as well as 2.0 ml of 7.5% sodium carbonate were subsequently added. The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 726 nm (JENWAY 6305). Gallic acid was used as the standard phenol and total phenols were expressed as gallic acid equivalents (GAE) (Singleton et al.,1999).

2.7 Determination of total flavonoid content

The total flavonoid content were determined by method given by Meda et al. (2005). For this 0.5 ml of aqueous extract mixed with 0.5 ml methanol, 50 μl of 10% AlCl3, 50 μl of 1 mol/L potassium acetate and 1.4 ml water and incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was measured at 415 nm using spectrophotometer here quercitin was used as standard.

2.8 Determination of vitamin C content

Vitamin C content of the extracted sample was determined by using the method given by Benderitter et al. (1998). Briefly, 75 μl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO4·5H2O in 100 ml of 5M H2SO4) were added to 500 μl reaction mixture (300 μl of an appropriate dilution of the extracts with 100 μl 13.3% trichloroacetic acid (TCA) and water). The mixture was incubated for 3 h at 37°C, thereafter 0.5 ml of 65% H2SO4(v/v) was added to the medium, and the absorbance was measured at 520 nm.

2.9 Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C18 column (4.6 mm x 150 mm) packed with 5μm diameter particles. As a mobile phase water containing 2% acetic acid (A) and methanol (B) was used, and the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 60 min, respectively, following the method described by (Amaral et al., 2013) with slight modifications.

All the samples were analyzed at a concentration of 20 mg/mL. Presence of eleven antioxidants compounds were identified. These compounds were performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.7 ml/min, injection volume 40 μl and the wavelength were 254 nm for gallic acid, 280 nm catechin and epicatechin, 327 nm for caffeic, ellagic acid and chlorogenic acids, and 365 nm for quercetin, quercitrin: isoquercitrin, kaempferol and rutin. The samples and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.020 – 0.200 mg/ml for quercetin, quercitrin isoquercitrin, kaempferol rutin, catechin and epicatechin; and 0.050 – 0.250 mg/ml for chlorogenic, ellagic acid, caffeic and gallic acids.

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The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 12563x + 1381.4 (r = 0.9991); caffeic acid: Y = 12738x + 1527.2 (r = 0.9999); ellagic acid: Y = 13084x + 1256.7 (r = 0.9997); chlorogenic acid: Y = 10972x + 1375.4 (r = 0.9998); rutin: Y = 11782 + 1460.3 (r = 0.9997); quercetin: Y = 12895x + 1342.5 (r = 0.9993); quercitin: Y = 11735x + 1439.6 (r = 0.9997); isoquercitrin: Y = 10982x + 1242.1 (r = 0.9996); kaempferol: Y = 13940 + 1173.9 (r = 0.9989); catechin: Y = 12194x + 1407.9 (r = 0.9995) and epicatechin: Y = 14176x + 1381.4 (r = 0.9991); chlorogenic acid: Y = 10972x + 1375.4 (r = 0.9998); quercetin: Y = 12895x + 1342.5 (r = 0.9993); quercitin: Y = 11735x + 1439.6 (r = 0.9997); isoquercitrin: Y = 10982x + 1242.1 (r = 0.9996); kaempferol: Y = 13940 + 1173.9 (r = 0.9989); catechin: Y = 12194x + 1407.9 (r = 0.9995) and epicatechin: Y = 14176x + 1381.4 (r = 0.9991). All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Sabir et al. (2012). LOD and LOQ were calculated as 3.3 and 10 times the standard deviation of the response and S is the slope of the calibration curve.

2.10 Data analysis

The results of the three replicate experiments were pooled and expressed as mean ± standard deviation (SD). Analysis of variance and the least significant difference test were carried out significance was accepted (P ≤ 0.05) (Zar 1984) followed by multiply comparison test (Tukey test) different letters differ by (P < 0.01) for HPLC- DAD characterizations of the phenolics.

3 Results

3.1 Biochemical traits evaluation

As shown in Table 1, ripening significantly increased the anti-nutrient phytate and tannin content of S. gilo fruits. Table 2 gives the proximate and nutrient composition of ripe and unripe S. gilo. The results showed that the carbohydrate range (35.89-36.96%) with no significant change (P > 0.05) in both samples.

| Table 1 Anti-nutrient content of unripe and ripe garden egg (S. gilo) . |
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| Table 2 Nutrient of unripe and ripe garden egg (Solanum gilo) % Proximate analysis. |

**Table 3 Mineral element (%) composition of unripe and ripe garden egg .**

[Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different (P < 0.05)].

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Amongst these phenolic constituents, gallic acid (ripe 49.8±0.01 mg g⁻¹ while unripe 50.34±0.01 mg g⁻¹) was the most abundant. Ripening brought about the present of ellagic acid (39.1±0.02 mg g⁻¹), quercitrin (39.6±0.02 mg g⁻¹), kaempferol (36.21±0.02 mg g⁻¹) which was not detected in the unripe. However there was a significant increased in isoquercitrin (34.2±0.02 mg g⁻¹), Caffeic acid (27.81±0.03 mg g⁻¹) Chlorogenic acid (38.51±0.02 mg g⁻¹) were relatively available as major phenolic compounds (Table 4). However, gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, isoquercitrin, quercitrin, quercetin, kaempferol and were identified and quantified through their retention time, as shown in Table 6, the major phenolic compound present is gallic acid.

4 Discussions

Phytic acid, considered as an antinutritional factor, is a common storage form of phosphorus in seeds, and in a few tubers and fruits. The complexing of phytic acid with nutritionally essential minerals and the possibility of interference with proteolytic digestion have been suggested (Oboh et al., 2005) to be responsible for the anti-nutritional activity. The phosphorus in phytic acid is not nutritionally available to the monogastric animals. Phytic acid also interferes with Ca, Fe, Mg and Zn absorption because of its ability to chelate divalent cationic minerals (Nelson et al.,1968) Although ripening increased the phytate and tannin of S.gilo when compared to unripe one but not much to have deleterious effect when compared the results to that of S. melogena leaves, bambara groundnut and some commonly know vegetables (Ijarotimi et al., 2010).

The results from this studies confirmed work done by Alkarkhi et al., 2010 on increased in physiochemical properties of banana peels. The calculated [phytate]/[Zn] molar ratio of ripe and unripe garden egg used for this studies were below 15.0 considered to be the critical value (Fergusson et al., 1988). This indicates that the phytate content will not reduce the bioavailability of zinc to a critical level in the calculated [Ca]/[phytate]. Wise (1983) has suggested that the solubility of phytate and proportion of zinc bound to the complex depend on the dietary Ca levels. In his model, phytate precipitation is not complete until dietary Ca/phytate molar ratios attain a value of approximately 6.0. At lower ratios, phytate precipitation is incomplete, causing some dietary zinc to remain in solution.However, the calculated [Ca]/[phytate]/[Zn] molar ratio is a better index for predicting Zn bioavailability due to a kinetic synergism which exists between [Ca] and [Zn] ions resulting in a Ca/Zn/phytate complex, which is less soluble than the phytate complex formed by either ion alone (Oberleas, 1973).

Table 4 Total phenols,Total flavonoids, ABTS*(2,2-Azinobis(3-ethylbenzo-thiazoline-6-sulfonate) scavenging ability,and vitamin C content of unripe and ripe garden egg (S. gilo).

| Samples | Total phenols (mg/100g) | Total flavonoids (mg/100g) | ABTS* (mmol/100gTEAC) | Vitamin C (mg/100g) |
|---------|------------------------|---------------------------|-----------------------|------------------|
| SGU     | 38.56±1.68^a           | 18.32±1.40^a              | 26.79±1.34^a          | 37.85±0.20^a     |
| SGR     | 44.28±1.77^b           | 20.73±1.48^b              | 44.63±1.45^b          | 30.15±0.10^b     |

[Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different (P <0.05)].
The result of the present study revealed that the calculated Ca/Zn/phytate molar ratios for the ripe and unripe garden egg were below the critical level. High concentration of tannins reduce satiety and nutrient digestibility, invariably low to moderate concentration improve the digestive utilization of feed which could be due to protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine (Schofield et al., 2001). These effects on nutrition are reflected in animal performance, however, this present showed that the value of tannin present in both samples from this studies is far below the critical level when compared with several studies done using different concentration of tannin source on animal model (Schofield et al., 2001; Frutos et al., 2004). Increase in tannin contents during ripening has been linked to their role as flavor contributors (Aina, 1990). This is associated with an increase in extractable flavonoids resulting from polymerization of tannins and other polyphenolic compounds. Moreso, an increased in tannin content during the ripening stages could also be attributed to slight decrease in macromolecules such as protein (Mamiro et al., 2007). This clearly shows that the ripe eggplant considered as waste could serve as a good combination with high protein diet for animals.

Table 5 I$_{50}$ value of DPPH scavenging ability (mg/ml).

| Compounds     | Ripe (mg/100g) | Unripe (mg/100g) |
|---------------|----------------|------------------|
| Gallic acid   | 49.8±0.01$^a$ | 50.34±0.01$^a$  |
| Catechin      | 10.61±0.01$^b$| 12.67±0.02$^a$  |
| Chlorogenic acid | 38.51±0.02$^c$| 20.71±0.01$^b$  |
| Caffeic acid  | 27.81±0.03$^d$| 39.46±0.03$^a$  |
| Ellagic acid  | 39.1±0.02$^f$ | ND               |
| Epicatechin   | 7.64±0.01$e$  | 14.53±0.02$^c$  |
| Rutin         | 18.72±0.01$^f$| 22.18±0.01$^b$  |
| Quercitin     | 39.6±0.02$^c$ | ND               |
| Quercetin     | 12.5±0.01$^f$ | 14.92±0.03$^b$  |
| Kaempferol    | 36.21±0.02$^h$| ND               |
| Isoquercitrin | 34.2±0.02$^c$ | 28.36±0.01$^e$  |

[Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.01$.]

According to work reported by Fonad (1996), a decreased in magnesium could be attributed to the conversion of chlorophyll, the green pigment in unripe garden egg, carotenoids, flavonoids and its derivatives that is responsible for the characteristic reddish colour of ripe garden egg, thus unripe garden egg have higher Mg content. It is apparent that ripe garden egg is a good source of potassium, but a poor source of sodium, calcium and iron.
Potassium has been reported to be the most predominant mineral in most tropical fruits, including garden egg (NWanna et al., 2013). It was evident in this study that the *Solanum gilo* ripe and unripe contained appreciable amounts of essential nutrients that maybe of nutritional and health benefits for humans and animals.

The positive health effects of phenolic phytochemicals is linked to their ability to counter the negative effects of reactive oxygen species generated during cellular energy metabolism. Flavonoids are also known as a class of widely distributed phytochemicals with antioxidant activities (Sies.,1986). *S. gilo* species are rich sources of phenolic phytochemical as depicted in Table 4 the phenols and flavonoids increased with ripening. Ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation. Moreover, studies have shown that ascorbic acid that is widely distributed in plant cells, a known antioxidant which reduced the effect of reactive oxygen species, it also acts as cofactor maintaining the activity of a number of enzymes, such as membrane bound antioxidant α-tocopherol in a reduced state (Arrigoni & De Tullio.,2002).

However, from the studies species vitamin C decreases with ripening from. Decrease in vitamin C could be attributed to its susceptibility to oxidative destruction (Aina,1990) as impacted by the ripening environment. The DPPH radical scavenging ability of garden egg extracts showed activities at a concentration dependent manner. Unripe had the strongest radical scavenging ability, compared to ripe samples (Figure 2; Table 5). DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule (Wolfe & Liu 2008). The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging (Cao et al.,1996).

There is agreement between the vitamin C and DPPH reported. DPPH is used in the determination of free radical scavenging ability of various food components; however, it has the limitation of color interference and sample solubility (Dorman & Hiltunen 2004). The total antioxidant ability of the extracts was further studied, using a moderately stable nitrogen- centered radical species; ABTS*. The ABTS* radical-based model of free radical scavenging ability has the advantage of being more versatile due to the minimal spectral interference, as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Re et al.,1999). The riped had the highest total antioxidant activity which could be attributed to its high phenols and flavonoids content.

Overall, the flavonoid composition is relatively increased than the phenolic acid present. Ellagic acid, 2,3,7,8-tetrahydroxy-chromeno is a powerful bioactive compound with many potential pharmacological and industrial applications (Sepulveda et al., 2011). Studies reported by Liang & Kitts (2015) revealed that chlorogenic acids can be used to control oxidative and inflammatory stress conditions, likewise, Vellosa et al. (2011) reported on the antioxidant and cytotoxic ability of Kaempferol, Quercetin and Isoquercitrin. Several reports have also established that these phenolic and flavonoids compounds are potent and effective antioxidants that possess free radical scavenging ability, reducing property and metal chelating effects, and are capable of inhibiting lipid peroxidation (Calderon-Montano et al.,2011). Phenolics and their metabolites are rapidly and widely distributed in organs and tissues in vivo after their consumption, where they can render their therapeutic effects. Passamonti et al. (2009), reported the bioavailability of phenolic compounds across the gastrointestinal epithelium and plasma circulation a few minutes after ingestion. Consumption of these ripe garden as part of livestock feeding stock instead of allowing it to rot and discarded away as a waste is rich in these phenolic and flavonoid compounds which may assist in maintaining consistent gastrointestinal and plasma concentrations, hence the overall wellbeing of the animal. Moreso it can be used in nutraceuticals.

**Conclusion**

In conclusion, ripe garden egg had lower nutrient and mineral composition as compared to unripe. However, ripe had higher antioxidant properties than the unripe. These results could not be far-fetched from the more polyphenols present in the colored flesh which was in accordance to the HPLC-DAD analysis. This study revealed that ripe garden egg is rich in phenolic phytochemicals

**Conflict of interest**

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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