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Comparative study of the bioactive and chemical properties of three different *Solanum* spp. from Ghana

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Abstract

The family *Solanaceae* represent one of the most economically, nutritionally and medicinally important families of angiosperms. The genus *Solanum* is a hyper-diverse taxon of this family. There are about 2300 species of *Solanum* in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the temperate areas. *Solanum torvum* has been extensively explored for its chemical constituents. To determine and compare the phytoconstituents of the fruits/berries of three different species of *Solanum (torvum*, *erianthum, and macrocarpon)* at different maturity stages. Fruits of *Solanum (torvum, erianthum, and macrocarpon)* were harvested at different maturity stages from local farms and around households in the Volta region and kept in sterile sample bags. Phytochemical and mineral analysis of the fruits was carried out according to the method of the Association of Official Analytical Chemists (AOAC), to determine the mineral composition of the fruits. Total phenolic content was determined by the Folin Ciocalteau method, Flavonoids content was determined by the Aluminum Chloride method. Antioxidant activity was determined by three assays; DPPH, FRAP, and ABTS. The elemental mineral analysis was done using the flame photometer according to methods by AOAC. The present study revealed that the levels of antioxidants, total phenolics, and flavonoids contents were significantly affected by both the physiological maturity stage and extraction solvents. Total phenolic content ranged between 16.97±0.03 - 40.62±0.00 µL GAE/10 µL. Flavonoids contents ranged from 52.33±0.36 - 434.00±0.31 µL CE/10 µL. Antioxidants activity values ranged between 37.79±036 - 878.34±4.36, 16.66±7.40 - 47.49±15.27 and 0.075±0.00-0.257±0.00 for DPPH, ABTS and FRAP assays respectively. Elemental mineral values recorded ranged between 4.87±0.03 - 11.55±0.05 mg/kg for Fe, 23.23±0.04 - 32.46±0.04 mg/kg for Mg and 34.317±0.03 - 15.3309±0.001 mg/kg for Na. This study revealed that the levels of antioxidants, total phenolics and flavonoids are significantly affected by both the physiological maturity stage and extraction solvents. It was clear that all methanolic extract showed significant variations with high values in antioxidants which included the DPPH scavenging activity, the $ABTS^{\bullet+}$ scavenging activity, the Ferric reduction power assay, the total phenolics, and the flavonoid content.

1. Introduction

Solanaceae is a plant family comprising of about 2300 species and nearly one-half belong to the genus *Solanum* (Oppong *et al*., 2015). The genus *Solanum* is comprised of about 1500 species and well represented all over the world. Its genomics in the genus *Solanum* is evolving at a moderate pace compared to other plant species (Oppong *et al*., 2015). It is rich in alkaloids which are distributed in all parts of the plant. The active components such as solinidine and other steroids extracted from the roots and leaves of some species are potent and effective pharmaceuticals. The majority of the *Solanum* species are widely used in folk medicine (Sundari *et al.*, 2013). Some example of species which belong to the genus *Solanum* are *ficifolium*, *ferrugineum*,

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torvum, erianthum, *indicum L, trilobatum, xanthocarpum*.

S. torvum is a common plant native to many tropical countries in Africa and the West Indies. It is also found in some parts of Asia. In Ghana and many other countries, the fruits are used for food. The leaves are used in many traditional medical practices in Africa (Nguelefack *et al.*, 2008). The plant grows as a short (about 2-4 m tall), erect shrub with lots of branches. *S. torvum* grows into a large thorny impenetrable thicket (Mohan and Bhandare, 2012). The fruits are employed in traditional medicine to cure cough, bronchial asthma, liver, and spleen enlargement. The fruits of *S. torvum* are clusters of tiny green spheres (about 1 cm in diameter) that become yellow when fully ripen. They are thin-fleshed and contain numerous flat, round, brown seeds (Akoto *et al*., 2015). In Ghana, *S. torvum* (locally referred to as 'Kwahu nsusua', 'Abeduro', 'Yaa Asantewaa', 'Kantosey' etc) is used essentially for food. In the south of Ghana, especially, it is added to palm nut soup and some stews (Akoto *et al*., 2015). *S. torvum* has both a sedative and a diuretic therapeutic effect. The leaves are used as a hemostatic. Phytochemical studies indicated that fruits of this species have good concentrations of various alkaloids, flavonoids, saponins, tannins, and glycosides which are sufficient to have pharmacological effects.

Solanum erianthum has been intentionally cultivated for food and medicine (Babalola *et al*., 2016). Like many members of the *Solanaceae* family, it contains bio constituents which are both highly toxic to humans and medicinally useful. This particular species possesses steroidal saponins and steroidal alkaloids that are useful in the pharmaceutical industry as steroidal precursors to produce anti-inflammatory corticosteroids, contraceptive steroids and anabolic steroids (Rasheed and Qasim, 2013). Essential oils in the fruits and leaves of *S. erianthum* have been studied for their traditional uses in medicine, especially for skin disease and stomach related ailment. The berries are cooked and eaten in Southeast Asia and made into curry in Southern India.

Solanum macrocarpon otherwise known as the African eggplant ("Gboma") belongs to the *Solanaceae* family (Haliński *et al*., 2019). It is a tropical biennial plant that is closely related to the eggplant; it grows to a height of 1-1.5 m and has an alternate leaf pattern with the blade width of 4 -15 cm and a height of 10 - 30 cm. The shapes of the leaves are oval and lobed with a wavy margin. The fruits are round, the top and the bottom are flattened out and have grooved portions with a length of 5 - 7 cm and a width of 7 - 8 cm. The stalk of the fruit is 1 - 4 cm long and is either curved or erect. At a young stage, the color of the fruit is green, ivory, or purple and white color with dark stripes. When ripe, the fruit turns yellow or a yellow-brown. The fruit contains many seeds and it is partly covered by the calyx lobes. The seeds have a length of 3-4.5 mm, a width of 2-3.5 mm, and the shape is obovoid. *S. macrocarpon* has a large cultivar and varieties that grow in areas of high rainfall found (Haliński *et al*., 2019).

The *Solanum* species are popularly known and consumed all over the world for their health benefits and nutritive values. In Ghana majority of the species especially *S. torvum* is consumed based on the knowledge that they have hematinic properties and promote overall health and wellbeing as well as *S. erianthum* which is popularly consumed in Asia and parts of West Africa (Kuffuor *et al.*, 2011). Though popularly known, little knowledge exists on the bioavailability of iron of these species. Locals have limited knowledge on the species that contain adequate and enough iron and even at different physiological maturity stages and hence tend to stick to one type especially with the fruits of *S. macrocarpon* which is underutilized due to lack of knowledge on its health benefits.

Several pieces of research conducted showed that turkey berries are a good source of iron. Extracts of the fruit possess high iron content and therefore justifying their use as hematinic (Akoto *et al*., 2015). Based on this knowledge, people are being advised to consume turkey berries without knowing which physiological maturity stage yields more nutrients and antioxidants, especially iron. Also due to lack of knowledge, only the leaves of *S. marcrocarpon* are consumed in certain parts of Ghana. This study aimed to determine and compare the mineral and phytochemical constituents of the fruits/berries of species of *Solanum (torvum*, *erianthum, and marcrocarpon)* at different physiological maturity stages.

2. Materials and methods

2.1 Study design

This study was a laboratory/bench work and employed a quantitative data collection method constituting various laboratory analyses of samples.

2.2 Samples and chemicals

2.2.1 Samples

Fruits/berries of *Solanum (torvum, erianthum, and macrocarpon*) (Figure 1) were sourced and harvested at different physiological maturity stages (early matured, matured and post matured) from farms and gardens in Ho and Tsito (Volta Region, Ghana) within the periods of September to December 2018.

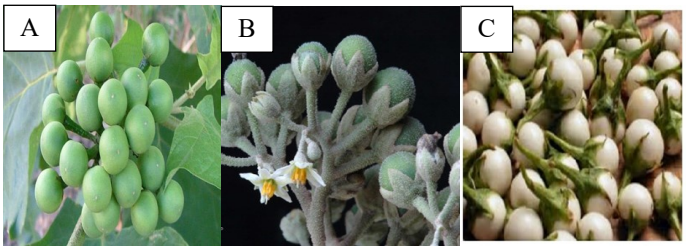

Figure 1. A- *Solanum torvum,* B - *Solanum erianthum,* C - *Solanum macrocarpon*

2.2.2 Chemicals

The chemicals and reagents used for analyzing the mineral and phytochemical contents included gallic acid, catechin, sodium nitrate, sodium carbonate, Folin Ciocalteu phenol reagent, trichloro-acetic acid, sodium nitrite, aluminium chloride, methanol, HCL, 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2, 4, 6-tripyridyl-S-triazine (TPTZ), $FeCl_3.3H_2O$, potassium persulphate, sodium acetate, Ascorbic acid and 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

2.3 Sample preparation

The samples were washed, sun-dried for a week and milled to a fine powder with a miller. The milled samples were then kept in a sterile sample bag for transport to study site and analysis. Samples were coded and labelled accordingly as follows:

St_1 = early/pre matured stage of *S. torvum*

St_2 = matured stage of *S. torvum*

St_3 = late/post matured stage of *S. torvum*

Se_1 = early/pre matured stage of *S. erianthum*

Se_2 = matured stage of *S. erianthum*

Se_3 = late/post matured stage of *S. erianthum*

Sm_1 = early/pre matured stage of *S. macrocarpon*

Sm_2 = matured stage of *S. macrocarpon*

Sm_3 = late/post matured stage of *S. macrocarpon*

2.4 Elemental mineral analysis

Standard solutions and aliquots of the diluted clear digest were used for flame photometry using the recommended standard methods of AOAC (2000) to determine the iron, magnesium and sodium content using atomic absorption spectrophotometry with protocols and standards established by the AOAC (2000).

2.5 Sample extraction

Ethanol (70%) and methanol (70%) were used for phenolic extraction. To 5 g of each dried powdered seed, 50 mL of ethanol (70%) and methanol (70%), was added in a 250 mL conical flask. The mixtures were shaken every 15 mins for 1 hr. The mixtures were then allowed to stand for 48 hrs with occasional shaking, after which the mixtures were filtered using 125 mm filter paper to remove solid particles. The filtrates were then transferred into pre-weighed Petri dishes and then evaporated at 50° C using a Heratherm oven to remove the ethanol and methanol. The extracts were then used to determine the total phenol content, total flavonoid, and antioxidant screening.

2.6 Determination of total polyphenol content

The total polyphenol content was calorimetrically estimated using Folin Ciocalteu reagent as described by Bhalodia *et al*., 2011) with modifications. About 50 g of each extract was dissolved in 1 mL of ethanol and vortexed. 5 mL of ethanol and 0.5 mL of Folin Ciocalteu's reagent were mixed and shaken. After 5 mins, 1.5 mL of 20% sodium carbonate was added and volume made up to 10 mL with ethanol. It was allowed to incubate for 2 hrs at room temperature. The intense blue color was developed. After incubation, absorbance was measured at 750 nm using a UV spectrophotometer (Jenway Vis Spectrophotometer). The blank was performed using reagent blank with solvent. Gallic acid was used as a standard. The calibration curve was plotted using standard garlic acid. The total phenol content of the berries of *Solanum* spp. was quantified and expressed as Gallic Acid Equivalent (GAE) mg/g sample weight basis (DW) and the range of a calibration curve was from 0.067 to 1.562.

2.7 Total flavonoid content

The total flavonoid content was determined by the aluminum chloride colorimetric assay with modifications. An aliquot of 5 g of the extracts was added to 10 mL test tubes containing 1mL of ethanol. About 150 µL of 5% sodium nitrite solution (5% $NaNO_2$) was added to each mixture and rested for 5 mins before the addition of 150 µL 10% aluminum chloride (10% $AlCl_3$). The resulting mixture was then allowed to stay for another 5 mins before adding 1 mL of 1 M sodium hydroxide (1.0M NaOH) and vortexed for 10 s. The absorbance of the aliquot was measured against prepared reagent blank at 510 nm and the total flavonoid content expressed as Catechin Equivalent (CE) mg/g on dry mass (Kalita *et al*., 2013).

2.8 Antioxidant activity

Spectrophotometric DPPH, ABTS and FRAP methods were used to determine the total antioxidant activity.

2.9 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH scavenging activities of both the crude extract and solvent fractions were determined as described by Kortei *et al*. (2014). A stock solution (1000 µg/mL) was prepared by dissolving 10 mg of each fraction in 10 mL of methanol. This was serially diluted to different concentrations (0, 10, 50, 100, 250, 500, and 1000 µg/mL). A 50 µL aliquot of each concentration was added to a mixture of 1000 µL of 0.1 mM DPPH and 450 µL of Tris buffer pH 7.4 and incubated for 30 mins at room temperature. The absorbance was determined at 517 nm using a VERSAmax microplate reader. The experiment was repeated twice and means values recorded. The radical scavenging activity was calculated and expressed as a percentage of the control (free radical solution minus plant extract) using the following formula:

$$\% \text{ Scavenging [DPPH]} = [(A_0-A_1)/A_0] * 100$$

Where A_0 was the absorbance of the blank (in which the same volume of methanol was used in place of the sample) and A_1 was the absorbance in the presence of the samples or standard.

Each compound with antioxidant activity should be extracted in at least one of the fractionation solvents. Hence, after the initial screening using the DPPH assay, the crude extract was excluded in the subsequent antioxidant assays.

2.10 Determination of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) acid (ABTS) scavenging activity

This was determined as per the protocol described by Re *et al.* (1999) with modification. A Stock solution of (7 mM, 10 mL) ABTS and (2.4 mM, 10 mL) potassium persulfate was prepared in distilled water and mixed to generate the ABTS free radical ($ABTS^{\bullet+}$). The resulting solution was incubated in the dark at room temperature for 12 hrs until the reaction was completed, by the observation of a constant absorbance. The $ABTS^{\bullet+}$ solution (1 mL) was further diluted in 50 mL of methanol and the absorbance calibrated to 0.7 at 734 nm. Dissolved extracts of 200 µL (prepared from 100 µL of extract dissolved in 900 µL of hexane) and standard (prepared from a stock solution of 1000 µg/mL in methanol) was added to 800 µL of the $ABTS^{\bullet+}$ solution, mixed and incubated at 30°C for 10 mins. The absorbance was read at 734 nm. The radical scavenging capacity was compared with that of BHA and the inhibition was calculated as a percentage of the control sample (free radical solution minus plant extract) as:

$$(\%) \text{ inhibition} = (\text{A control} - \text{A sample})/\text{A control} \times 100$$

Where A control is the absorbance of $ABTS^{\bullet+}$ solution and A sample is the absorbance of $ABTS^{\bullet+}$ sample (extract /standard). Negative control was prepared by replacing each fraction or standard with an equal volume of methanol.

2.11 Ferric reducing antioxidant potential (FRAP) assay

Ferric reducing power of fruits of *Solanum (torvum, erianthum, and macrocarpon*) extracts were determined using FRAP assay as per the protocol described by Luqman *et al*. (2012). This method is based on the reduction of colorless ferric complex (Fe^{3+} tripyridyltriazine) to blue-colored ferrous complex (Fe^{2+} tripyridyltriazine) by the action of electron-donating antioxidants at low pH. The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and with 1 volume of 20 mM ferric chloride. All the required solutions were freshly prepared before their uses. 100 µL of samples (µL/mL) was added to 3 mL of prepared FRAP reagent. The reaction mixture was incubated in a water bath for 30 mins at 37°C. Then, the absorbance of the samples was measured at 593 nm. The difference between the absorbance of the sample and the absorbance of blank was calculated and used to calculate the FRAP value.

2.12 Statistical analysis

The experiment was carried out in duplicates and values were expressed as means and standard deviations. One- way analysis of variance (ANOVA) was done using IBM Statistical Package for Social sciences (SPSS) version 2.0. Means were separated using Duncans Multiple Range Test (DMRT), significance was denoted at 5% in all instances.

3. Results

3.1 Sample extracts and percentage yields

Table 1 shows the extraction yield of the dried and milled fruits/berries of *Solanum (torvum, erianthum*, and *macrocarpon*) using 70% methanol. Methanol extracts of fruits of *S. torvum* at the 3 different ripening stages produced the highest yield as compared to *S. erianthum* and *S. marcrocarpon*. The methanol extract of the post matured stage of *S. torvum* recorded the highest percentage yield with 21.40% of all maturity stages of *S. torvum*. This was followed by the pre/early matured stage of *S. erianthum* with 11.40% yield and finally, the pre/early matured stage of *S. macrocarpon* had the third-highest yield. The percentage yield of *Solanum (torvum, erianthum*, and *macrocarpon*) at different maturity stages using methanol extract decreased in the following

Table 1. Percentage yield of dried powdered fruits in methanol solvent

Sample ID	Mass of empty petri dish (g)	Mass of empty petri dish + extract (g)	Mass of extract (g)	Percentage yield (%)
St_1	44.3	44.84	0.54	10.8
St_2	48.96	49.46	0.5	10
St_3	45.3	46.37	1.07	21.4
Se_1	41.96	42.53	0.57	11.4
Se_2	42.5	43.02	0.52	10.4
Se_3	42.01	42.32	0.31	6.2
Sm_1	42.69	43.26	0.57	11.4
Sm_2	41.96	42.24	0.28	5.6
Sm_3	40.08	40.35	0.27	5.4

order: late/post matured stage of *S. torvum* (St_3) > early/ pre matured stage of *S.erianthum* (Se_1) > early/pre matured stage of *S. macrocarpon* (Sm_1) > early/pre matured stage of *S.torvum* (St_1) > matured stage of *S. erianthum* (Se_2) > matured stage of *S. torvum* (St_2) > late/ post matured stage of *S. erianthum* (Se_3) > matured stage of *S. macrocarpon* (Sm_2) > late/post matured stage of *S. macrocarpon* (Sm_3).

3.2 Evaluation of antioxidant activity

In order to ascertain the antioxidant activity, the extracts (with methanol and ethanol) were screened using three different assays which included DPPH (1, 1-diphenyl–2 picrylhydrazyl) radical scavenging activity, ABTS [(2,2-azinobis,3-ethylbenzothiazoline-6-sulfonic acid)] and Ferric Reducing Antioxidant Potential (FRAP). Results of DPPH are shown in Table 2. Methanol extracts recorded a range of 37.79±0.36 - 88.34±4.36 for *S. erianthum* (Se_3, Se_1). Generally, extraction with methanol was statistically different ($p<0.05$) among the various stages of *S. torvum* and *S. erianthum*. However, values obtained for *S. macrocarpon* were comparable ($p>0.05$).

Ethanol recorded a ranged of 6.97±2.09 - 39.53±1.74 for *S. macrocarpon* (Sm_3) *S. torvum* (St_2). There were statistically significant differences ($p<0.05$) observed among the different stages of *S. torvum* and *S. macrocarpon* while no significant differences ($p>0.05$) for *S. macrocarpon*.

There was a decreasing trend DPPH radical scavenging activity with the increasing maturity time of the fruits.

3.3 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) acid (ABTS) radical scavenging activity

ABTS is used to measure the antioxidant capacities of food. In this assay, ABTS is converted to its radical cation by the addition of sodium persulfate. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols, and vitamin C. During this reaction the blue ABTS radical cation is converted back to its colorless neutral form. Results showed a methanol range of 16.66±7.40 - 47.49±15.27 (Se_3, St_2) and showed a significant difference ($p<0.05$) in all maturity stages of all species while ethanol ranged between 1.61±0.64 - 20.60±0.18 (Sm_3, St_3). All species showed significant differences ($p<0.05$) except for *S. erianthum* (Table 3).

3.4 Ferric reducing antioxidant power based on FRAP assay

FRAP assay measures the reducing potential of an

Table 2. DPPH radical scavenging activity of fruits of *Solanum* (*torvum, erianthum* and *macrocarpon*) extract

Sample ID	Methanolic Extraction (%)	Ethanolic Extraction (%)
St_1	$45.64±2.57^c$	$9.10±1.46^a$
St_2	$68.16±16.30^d$	$39.53±1.74^c$
St_3	$71.54±9.38^c$	$28.87±4.12^b$
Se_1	$88.34±4.36^d$	$28.68±1.46^b$
Se_2	$72.82±15.88^c$	$27.5I±2.20^b$
Se_3	$37.79±0.36^c$	$25.77±2.04^b$
Sm_1	$60.93±13.42^c$	$29.06±0.58^b$
Sm_2	$78.34±0.63^c$	$18.79±2.92^b$
Sm_3	$57.53±9.58^c$	$6.97±2.09^a$

Values are means±SD of 3 replications. Means in a column with the same superscript are not significantly different ($p\geq0.05$).

Table 3. ABTS radical scavenging activity of extracts of *Solanum* (*torvum, erianthum* and *macrocarpon*) extract

Sample ID	Methanolic Extraction (%)	Ethanolic Extraction (%)
St_1	$37.22±12.53^c$	$6.14±0.32^a$
St_2	$47.49±15.27^d$	$12.08±7.29^b$
St_3	$45.64±3.93^d$	$20.60±0.18^b$
Se_1	$40.00±21.79^d$	$14.45±1.86^b$
Se_2	$46.38±8.17^d$	$15.42±3.27^b$
Se_3	$16.66±7.40^b$	$18.23±0.49^b$
Sm_1	$47.22±17.97^d$	$17.47±2.33^b$
Sm_2	$37.49±15.43^c$	$10.89±6.16^b$
Sm_3	$35.27±18.33^c$	$1.61±0.64^a$

Values are means±SD of 3 replications. Means in a column with the same superscript are not significantly different ($p\geq0.05$).

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antioxidant reacting with a ferric tripyridyl triazine (Fe^{2+}-TPTZ). At a low pH of about 3.6, the reduction of Fe3+-TPTZ complex to blue colored Fe^{2+}-TPTZ takes place which has an absorbance at 593nm. At equal concentrations of different extraction solvents, there was an indication of the difference in the ferric reduction activity of the extracts. Comparing the different extraction solvents, ethanol recorded the highest solvent with the ferric reducing activity was followed by methanol. Extraction with ethanol the species that recorded the highest antioxidant activity of 0.607±0.00 using the FRAP assay was the post/late matured stage of *S. macrocarpon* followed by the pre/early matured stage of *S. torvum* (0.162±0.00) while the pre/early matured stage of *S. macrocarpon* produced the least antioxidant activity of 0.023±0.00. Also comparing the results for methanolic extraction, it was noted that the matured stage of the fruits of *S. erianthum* produced the highest antioxidant activity of 0.257±0.00 followed by the late/ post matured stage of the same *S. erianthum* (0.252±0.00). The species that recorded the third-highest value was the pre/early matured stage of *S. torvum* (0.230±0.00) with the matured stage of the same *S. torvum* (0.075±0.00) recording the lowest or least value. Table 4 shows the reducing power activity of *Solanum* (*torvum, erianthum*, and *macrocarpon*) at different maturity stages using different extraction solvents. Ferric reducing the power of extracts did not vary significantly among maturity/ripening stages the solvent used for extraction. The highest ferric reducing power of 0.607±0.00 was recorded by the ethanol extract of the post/late matured stage of *S. macrocarpon*. It was also observed that for *S. torvum*, the ferric reducing activity was not consistent as berries matured but activity varied significantly depending on the maturity stage for both ethanol and methanol extraction. Subsequently, with *S. erianthum,* ferric reducing activity did not show consistency in increase as fruits/berries matured but rather activity reduced as the fruits/berries are at the post/late maturity stage for both methanol and ethanol extracts. For both solvents, the matured stages recorded the highest values (0.257±0.00, 0.141±0.00). Accordingly, a similar trend was also observed in *S. macrocarpon*. Ferric reducing activity of *S. macrocarpon* showed significant variation ($p<0.05$) among the maturity stages. There was an observed irregular pattern of distribution of FRAP values as maturity progressed among the different species of *Solanum*.

Table 4. Ferric reducing antioxidant potential of extracts of *Solanum (torvum, erianthum and macrocarpon*) against ascorbic acid (positive control)

Sample ID	Methanolic Extraction (%)	Ethanolic Extraction (%)	Ascorbic Acid (%)
St_1	0.230±0.00^{d}	0.162±0.00^{c}	
St_2	0.075±0.00^{b}	0.061±0.00^{a}	
St_3	0.126±0.00^{c}	0.118±0.00^{b}	
Se_1	0.172±0.00^{b}	0.045±0.00^{a}	
Se_2	0.257±0.00^{c}	0.141±0.00^{b}	2
Se_3	0.252±0.00^{c}	0.136±0.00^{b}	
Sm_1	0.092±0.00^{a}	0.023±0.00^{a}	
Sm_2	0.202±0.00^{c}	0.152±0.00^{b}	
Sm_3	0.161±0.00^{b}	0.607±0.00^{d}	

Values are means±SD of 3 replications. Means in a column with the same superscript are not significantly different ($p\geq0.05$).

3.5 Total phenolic and flavonoid content

3.5.1 Total phenolic content

The total phenolic content of *Solanum (torvum, erianthum, and macrocarpon*) extracts of both methanol and ethanol solvents was measured and expressed as mg of gallic acid (standard phenolic compound) per gram dry weight of extract (µL GAE/10 µL) (Table 5). Using methanol as the extraction solvent had the highest phenolic content. The ethanol extract of matured stage of *S. macrocarpon* (25.28±0.25 µL GAE/10 µL) recorded the highest followed by the post matured stage of the same *S. macrocarpon* (23.86±0.19 µL GAE/10 µL) and pre/early matured stage of *S. torvum* recorded the least (13.19±0.01 µL GAE/10 µL). With the methanol extract, the matured stage of *S. erianthum* (40.62±0.00 µL GAE/10 µL) recorded the highest phenolic content followed by the pre/early matured stage of *S. macrocarpon* (40.61±0.00 µL GAE/10 µL) and the post/ late matured stage of *S. torvum* (16.97±0.03 µL GAE/10 µL) recorded the least value.

Table 5. Total phenolic content of extracts of *Solanum* (*torvum, erianthum* and *macrocarpon*) extract

Sample ID	Methanolic Extraction (µL GAE/10 µL)	Ethanolic Extraction (µL GAE/10 µL)
St_1	18.09±0.00^{b}	13.19±0.01^{a}
St_2	19.70±0.00^{b}	14.42±0.14^{a}
St_3	16.97±0.03^{b}	13.29±0.15^{a}
Se_1	26.95±0.00^{c}	16.91±0.10^{b}
Se_2	40.62±0.00^{d}	16.95±0.07^{b}
Se_3	29.94±0.07^{c}	22.69±0.19^{c}
Sm_1	40.61±0.00^{d}	23.51±0.37^{c}
Sm_2	22.15±0.65^{c}	25.28±0.25^{c}
Sm_3	26.05±0.00^{c}	23.86±0.19^{c}

Values are means±SD of 3 replications. Means in a column with the same superscript are not significantly different ($p\geq0.05$).

3.5.2 Total flavonoids content

The total flavonoid content of the crude extract and various fractions was measured and expressed as mg of quercetin (standard flavonoid compound) per gram dry weight of the extract. Range of values recorded for

methanol was 96.25±0.36 - 434.55±0.31 µL CE/10 µL (St_1, Sm_3, and Se_3). *S. erianthum* produced the greatest quantity of flavonoids which was significantly ($p<0.05$) different from all the methanolic extracts. For ethanol, a range of 109.54±0.30 - 784.54±0.30 µL CE/10 µL (St_1, Sm_3, and Se_2). Generally, greater quantities of ethanolic extracts (>100 µL CE/10 µL) of the flavonoids were obtained in this study with *S. erianthum* producing the maximum (Table 6).

3.6 Mineral composition

The elemental mineral composition (iron, potassium and magnesium content) of dried and milled fruits/ berries of *Solanum (torvum, erianthum, and macrocarpon)* are presented in Table 7. From the table, it was observed that the Post/late matured stage of *S. torvum* recorded the highest iron content of 11.55 mg/kg followed by the matured stage of the same *S. torvum* (11.21 mg/kg). There was no significant difference ($p>0.05$) between late/post matured and matured stages. However, the pre/early matured stage of *S. torvum* recorded the least iron content of 6.32 mg/kg and was significantly ($p<0.05$) lower. With magnesium, the recorded range of values was 24.88±0.11- 32.46±0.04 mg/kg for St_1 and Se_3 respectively. There were generally statistical differences ($p<0.05$) observed. *S. torvum* recorded a generally low level of Mg (24-26 mg/kg). Lastly, sodium recorded a range of 11.50±0.04 - 41.82±0.16 mg/kg for Sm_3 and Se_2. Notably, the results showed significant differences ($p<0.05$) between all samples analyzed (Table 7).

4. Discussion

All over the world, plants of the family *Solanaceae* have been employed for their edible and pharmaceutical benefits. In Ghana, locals depend heavily on the leaves and fruits/berries of most *Solanum* species for their mineral and antioxidant supplementations. Antioxidants are protective substances that protect the body's cells against the effects of free radicals and some naturally occurring antioxidants include flavonoids, tannins, phenols, and lignans-based foods are the best sources of antioxidants. Extraction is the main process by which bioactive compounds may be obtained from biomass materials. The objective of the extraction process is to maximize the number of target compounds and obtain the highest biological activity of these extracts (Truong *et al*., 2019). The extraction yield and biological activity of the resulting extract are not only affected by the extraction technique but also by the extraction solvent (Pour *et al*., 2011).

Variations observed for the extracts of the different samples could be attributed to differences in the polarity of the extraction solvents which might have caused a wide variation in the level of bioactive compounds in the extracts (Truong *et al*., 2019).A higher extraction yield was observed in the methanolic extract as compared to that of the ethanol extract (data not shown) indicating that the extraction efficiency favors the highly polar solvent which is methanol than ethanol which is less polar.

Antioxidant capacity is an important parameter that is used to establish the health benefits of food and food products and represent the ability to inhibit the process of oxidation (Bhandari and Lee, 2016). It is a very beneficial and desirable property of foods as oxidation by free radicals plays a major role in the pathogenesis of many human diseases as well as aging. Different *Solanum* species exhibit variation in their antioxidant activity of which *S. torvum, S. erianthum*, and *S. macrocarpon* are of no exception. This could be due to the presence of several natural compounds such as tannins, saponins, lycopene, phenolic compounds, flavonoids, etc. (Martínez-Valverde *et al*., 2002). The

Table 6. Total flavanoid content of extracts of *Solanum* (*torvum, erianthum* and *macrocarpon*) extract

Sample ID	Methanolic Extraction (µL CE/10 µL)	Ethanolic Extraction (µL CE/10 µL)
St_1	96.25±0.36^{a}	109.54±0.30^{b}
St_2	247.39±0.03^{b}	434.22±0.15^{c}
St_3	52.33±0.46^{a}	154.60±0.38^{b}
Se_1	134.51±0.26^{b}	426.28±0.40^{c}
Se_2	114.22±0.14^{b}	784.54±0.30^{d}
Se_3	434.55±0.31^{c}	286.16±0.22^{b}
Sm_1	134.49±0. 38^{b}	479.51±0. 26^{c}
Sm_2	62.29±0.38^{a}	472.63±0.04^{c}
Sm_3	96.25±0.36^{a}	109.54±0.30^{b}

Values are means±SD of 3 replications. Means in a column with the same superscript are not significantly different ($p\geq0.05$).

Table 7. Mineral content of dried fruits/berries of *Solanum (torvum, erianthum and macrocarpon)*

Sample ID	Fe (mg/kg)	Mg (mg/kg)	Na (mg/kg)
St_1	6.32±0.05^{b}	24.88±0.11^{b}	15.33±0.01^{g}
St_2	11.21±0.02^{e}	24.93±0.04^{b}	34.32±0.03^{a}
St_3	11.55±0.07^{e}	25.60±0.14^{c}	39.27±0.56^{c}
Se_1	6.33±0.03^{b}	31.22±0.17^{f}	56.91±0.04^{e}
Se_2	4.87±0.03^{a}	23.23±0.04^{a}	41.82±0.16^{d}
Se_3	10.65±0.06^{d}	32.46±0.04^{g}	36.62±0.09^{b}
Sm_1	8.55±0.06^{c}	29.58±0.12^{d}	35.03±0.09^{a}
Sm_2	10.09±0.00^{d}	30.66±0.23^{e}	34.82±0.02^{a}
Sm_3	8.35±0.06^{c}	30.83±0.02^{e}	11.50±0.04^{f}

Values are means±SD of 3 replications. Means in a column with the same superscript are not significantly different ($p\geq0.05$).

DPPH assay is known to be a rapid, simple and inexpensive way to determine the antioxidant activity of food and food products by testing their ability to act as free radical scavengers or hydrogen donors (Bhandari and Lee, 2016).

DPPH is a nitrogen-centered free radical; hence any compound that can scavenge a significant amount of DPPH may be useful in reducing the levels of other reactive nitrogen species in living cells (Tettey *et al*., 2014). The basis of this method is that antioxidants react with the stable free radical (DPPH) and convert it to 2,2-diphenyl-1-picrylhydrazyl, which is accompanied by a color change from purple to yellow (Bhandari and Lee, 2016). Among the extracts tested, the methanolic extract was the most potent in terms of values of DPPH scavenging activity. Remarkably, methanolic extract of all samples exhibited a two-fold higher DPPH scavenging activity than that of ethanolic extracts. This could be as attributed to the reason that this extract contains the highest level of phenolics, flavonoids, alkaloids, and terpenoid compounds, etc. Those compounds possess powerful antioxidant activity and subsequently protect the human body against oxidative damage. Also, from the results, it was evident that DPPH radical scavenging activity of species varied significantly at different maturity/ripening stages. It was obvious from the table that the pre/early matured stage of *S. erianthum* recorded the highest activity followed by the matured stage of *S. macrocarpon* and finally the late/post matured stage of *S. torvum*. The mean yield of ethanol extract of methanol was different from the findings of (Batra and Sharma, 2013). This could be attributed to the presence of other compounds like saponins, tannins, and alkaloids which increases or decreases as fruits/berries mature.

$ABTS^{\bullet+}$ is a protonated free radical and hence was used in this study to examine the capacity of the leaf extracts to reduce the positive charged radical in solution. This assay was also included in this study because $ABTS^{\bullet+}$ is sensitive over a broad range, from highly potent to very weak antioxidants, the latter which may not be detected by the DPPH assay. Compounds with one OH group in the aromatic ring which are found inactive towards the DPPH radical are significantly active towards $ABTS^{\bullet+}$ (Nenadis and Tsimidou, 2002). It was observed from Table 4 that the $ABTS^{\bullet+}$ scavenging activity varied significantly with both methanol and ethanol extraction. Values for methanolic extract recorded higher values than that of the ethanolic extract and values also showed variation at different maturity stages. For *S. macrocarpon*, it was observed that $ABTS^{\bullet+}$ scavenging activity for both ethanol and methanol increased as fruit/berries matured from one stage to another. Subsequently, for *S. torvum* and *erianthum* (methanol), activity did not show consistency from one maturity stage to another.

The assessment of antioxidant activity to reduce iron represents the ability of a substance to transfer an electron or hydrogen atom from another substance to and an antioxidant ability to reduce the oxidized intermediates during the peroxidation process (Gómez-Ordóñez *et al*., 2012). All the variations were different from the findings of Gandhiappan and Rengasamy, (2012) and could be attributed to the presence of phenolic compounds that may act by donating the electrons and acting with free radicals to convert them to more stable products and terminate radical chain reaction (Du *et al*., 2012).

Phenolic compounds are very important metabolites and perform and possess various biological functions of which the most important is an antioxidant activity associated with the reduction of cancer and cardiovascular diseases (Toor and Savage, 2005). Phenolic compounds contribute about 60-70% of the antioxidant activity in *Solanum* species. In this study, the total phenolic content in the extract of the three *Solanum* species showed independent patterns at different physiological maturity stages. Phenolic content showed variation in increment as fruits/ berries got to different maturity stages. The results of this study disagreed with the research findings of Waghulde *et al*. (2011) who reported values of 9.953 mg GAE/100 g and 7.890 GAE/100 g respectively for methanol and ethanol extracts of *S. torvum* phenol content. Such fluctuations in the total phenolic content could be mainly due to the differences in several factors such as temperature, sunlight, rainfall and compounds such as tannin, saponins, etc. that contributed to the variations. However additional studies are needed for further clarification.

Flavonoids are secondary plant metabolites that possess strong antioxidant, an anti-proliferative and antibacterial activity which are known to increase with plant stress (Panche *et al*., 2016). They play an important role in the control of plant resources to biotic and abiotic stress. In human nutrition, flavonoids are considered as potential health-promoting substances due to their anti-oxidative, anti-cancer, and cardiovascular protective effects. They also have anti-microbial, anti-inflammatory, anti-aging and neuroprotective effects (Ginwala *et al*., 2019). In this present study, the flavonoid content of both methanol and ethanol extract as seen in Table 4 indicated that there was a significant variation in flavonoid at the different maturity stages and values for both methanol and ethanol extract were significantly different. It was observed that *S. torvum* showed inconsistency in flavonoid content as the fruits/

berries moved from one physiological maturity stage to another. Finally, it was interesting to note that flavonoid content was directly proportional to the maturity stage. Variation in flavonoid content in fruits is strongly influenced by extrinsic factors such as fruit type and growth, season, climate, degree of ripeness, food preparation, and processing as suggested by Lakenbrink *et al*. (2000) and Panche *et al*. (2016).

Overproduction of free radicals i.e oxidative as well as nitrosative stress during hypercholesterolemia is a major root reason for the pathophysiology of atherosclerosis and other related cardiovascular diseases (Lahera *et al*., 2007). The importance of dietary phenolic compounds as highlighted by Chiu *et al.* (2018) in combating cardiovascular diseases cannot be overemphasized. Consumption of *Solanum* spp. is very beneficial for human health as it has been proven to possess adequate amounts of essential phytochemicals.

4.1 Mineral composition

Minerals are vital elements required for the normal growth and maintenance of the body. They are also important constituents of the human diet as they serve as co-factors for many physiological and metabolic processes (Arivalagan *et al*., 2013). Deficiencies of micro-minerals are a major global health problem. More than 2 billion peoples in the world today are estimated to be deficient in key minerals particularly minerals (Arivalagan *et al*., 2013). The significance of these elements cannot be overemphasized.

Iron is an indispensable mineral in the body that is involved in processes such as hemoglobin production and oxygenation of red blood cells, digestion and circulation among many others are carried out in the human body as suggested by Agyei-Poku (2018). For its function in hemoglobin production, it's mostly required in the diet of pregnant women, nursing mothers, infants, convulsing patients and the elderly to prevent anemia and other related diseases (Bamishaiye *et al*., 2011). The results indicated that iron was the least concentrated of all the minerals analyzed in the samples. Also, the iron content of both *S. erianthum* and *macrocarpon* did not follow a similar trend as fruits/berries moved from one maturity stage to another. Dissimilar results were reported by Akoto *et al*. (2015), Mahapatra *et al*. (2012) and Otu *et al*. (2017). However, higher values were recorded by Eletta *et al*. (2017) and Sam *et al*. (2012). The differences in iron content among maturity stages of different species could be attributed to factors such as type of fruit, degree of ripeness climate, etc and also the presence of other phytochemicals.

Magnesium is an important co-factor of many regulatory enzymes, particularly the kinase, and is fundamental in the energy transfer reaction involving high energy compounds like ATP and creatinine phosphate and thus muscle contraction (Brewer and Wallimann, 2000). Magnesium is involved in enzymatic reaction of carbohydrate catabolism e.g. glycolysis. Its deficiency in man, however, may lead to severe diarrhea, migraines, hypertension, cardiomyopathy, arteriosclerosis and stroke (Bamishaiye, 2011). Values obtained in this study disagreed with values reported in a study by Agyei-Poku (2018) of a range of 1365-5550 mg/Kg. This observation indicated that magnesium concentrations were not directly proportional to the maturity stages but rather this variation could be attributed to different factors that affect magnesium concentrations in fruits. The values obtained in these studies are low to meet the Recommended Daily Allowance (RDA) of 400 mg/100 mL for men, women of 19 to 39 years old (Institute of Medicine (US) Standing Committee on the Scientifice Evaluation of Dietary Reference Intakes, 1997).

Sodium helps in the transmission of nerve impulses and brings about an osmotic balance of the cells in the living tissue.

5. Conclusion

In conclusion, the present study revealed that the levels of antioxidants, total phenolics and flavonoids are significantly affected by both the physiological maturity stage and extraction solvents. It was clear that all methanolic extract showed significant variations with high values in antioxidants which included the DPPH scavenging activity, the $ABTS^{\bullet+}$ scavenging activity, the ferric reduction power assay, the total phenolics, and the flavonoid content. It was also interesting to note that most values for all these phytochemical constituents varied drastically as the physiological maturity stage varied. In general, some values reduced down the stages that are from the early/pre matured stage to the post/late matured stage and others increased down the stages. Some species did not also show consistency in increment or decrement of values down thee physiological maturity stages. Also, the three species contained significant amounts of the minerals iron, magnesium, and sodium at different maturity stages. Some of the values obtained were lower when compared with the Standard Dietary Allowance (RDA) and others too were higher.

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