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Morphological characteristics, bioactive compounds content, and antioxidant activity of different accessions of African eggplant (*Solanum anguivi* Lam.)

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Summary

African eggplant *(Solanum anguivi* Lam.) fruits reportedly exhibit antidiabetic properties, possibly due to the presence of bioactive compounds. This study aimed to assess the bioactive compounds content (BCC) and antioxidant activity (AA) in the fruits of fourteen African eggplant accessions. The relationship between the fruit BCC and AA, and the plant (leaf, stem and fruit) morphological characteristics was determined. Morphological traits for the plant accessions were characterized based on existing *Solanum* species descriptors. Total phenolics, flavonoids, saponins, vitamin C and AA were determined by spectrophotometry, while total alkaloids were detected by gravimetry. HPLC was used for the quantification of phenolic compounds. Morphological characteristics, BCC and AA differed among the accessions. The fruit's accessions contained total phenolics (8.0-12.4 mg gallic acid equivalent/g dry weight (DW)), saponins (51.1-124.8 mg diosgenin equivalent/g DW), alkaloids (81.4-127.7 mg/g DW), vitamin C (3.6-6.4 mg ascorbic acid equivalent/g DW), and flavonoids (0.9-2.1 mg quercetin equivalent (QE)/g DW) and exhibited a high AA (1.2-4.6 mg QE/g DW). Amongst the quantified phenolic compounds, chlorogenic acid (21.4-301.3 µg/g DW) had the highest content. Cluster analyses showed that morphological characteristics might be useful to predict accessions with similar BCC and AA. Accessions with high total phenolics provided the highest AA, and, therefore, may mediate health benefits.

Keywords: *Solanum anguivi*, antioxidant capacity, bioactive compounds, morphological characteristics, accession.

Introduction

Fruits and vegetables are particularly protective against diseases associated with oxidative stress due to possessing bioactive compounds such as phenolics, flavonoids and saponins (ZHANG et al., 2015). An imbalance between overproduction or accumulation of free radicals in the body and the potential of a biological system to detoxify the reactive substances results in oxidative stress (DHALARIA et al., 2020). Oxidative stress leads to the damage of large biomolecules such as lipids, DNA, and proteins, resulting in the pathogenesis of non-communicable diseases (NCDs) such as type 2 diabetes mellitus (T2DM), as evidenced in several clinical and experimental studies (MARITIM et al., 2003; ZHOU et al., 2003; ZHANG et al., 2015). Collectively, NCDs (cardiovascular diseases, cancer, diabetes, and chronic respiratory disease) are responsible for 71% of all deaths worldwide (WHO, 2018). Plant bioactive compounds with antioxidant properties play a vital part in reducing oxidative stress by inhibiting free radical inhibitors, which ultimately prevents and/ or treats NCDs (DHALARIA et al., 2020)

Solanum anguivi Lam. fruits (SALF) may alleviate diseases such as hypertension, atherosclerosis, and diabetes (ELEKOFEHINTI et al., 2013a). This may be attributed to bioactive compounds present in the fruits such as phenolics, flavonoids, saponins, and alkaloids (ELEKOFEHINTI et al., 2013b; OYEYEMI et al., 2015). *Solanum anguivi* Lam. belongs to the family *Solanaceae* and genus *Solanum* L. (BUKENYA and CARASCO, 1995; UNITED STATES DEPARTMENT OF AGRICULTURE, 2021). It is native to Africa and has also been reported to occur in Asia and Australia (BUKENYA and CARASCO, 1995; JAYANTHY et al., 2016; NAKITTO et al., 2021). It is commonly known as "forest bitter berry" or "African eggplant", although the latter is also used in reference to *Solanum aethiopicum* (*S. aethiopicum)* and *Solanum macropcarpon* (*S. macropcarpon)* (ELEKOFEHINTI et al., 2013a). *Solanum anguivi* Lam. (*S. anguivi* is henceforth used in reference to the whole plant) plants are consumed as leafy and/ or fruit vegetables (DENTON and NWANGBURUK, 2011).

The bioactive compounds content (BCC) and morphological characteristics of some *Solanum* fruits have been reported to vary among their accessions (HANSON et al., 2006; SSEREMBA et al., 2017; TEMBE et al., 2020). Although the BCC and antioxidant capacity (AA) of African eggplants have been reported (ELEKOFEHINTI et al., 2013b; OYEYEMI et al., 2015), there is paucity of data on their variations among the accessions. Some studies (OSEI et al., 2010; SSEREMBA et al., 2017; TEMBE et al., 2020) have reported variations in some morphological (flower, stem, fruit, and leaf) characteristics in *S. anguivi* accessions. However, data on variations in other morphological characteristics among *S. anguivi* accessions as well as the relationship between morphological characteristics and, BCC and AA are lacking. This information would equip researchers and consumers with the knowledge to identify *S. anguivi* accessions better, and to possibly predict the accessions with similar BCC and AA based on their morphological characteristics.

This study sought to assess the BCC and AA of fruits of *S. anguivi* accessions and to determine whether these were related to the plant morphological characteristics. Additionally, the study determined the relationship between the BCC and AA of the fruits. Regression models were derived to determine the BCC that may mostly affect the AA of the fruits.

Materials and methods

Chemicals and reagents

The reference standards (quercetin, gallic acid, L-ascorbic acid, diosgenin), HPLC grade -methanol, ethanol, sulphuric acid, acetic acid, ammonium hydroxide; and analytical grade -thiourea, trichloroacetic acid, Folin-Ciocalteu reagent, 1-diphenyl-2-picrylhydrazyl (DPPH), vanillin, dinitrophenyl hydrazine, hydrated copper (II) sulphate, potassium acetate, sodium bicarbonate, and aluminium chloride, were all from Sigma-Aldrich (Munich, Germany).

Plant collection

A preliminary survey was carried out to determine the *S. anguivi* accessions in Mukono district (Uganda), where they have been documented to grow (STEDJE and BUKENYA-ZIRABA, 2003). Nabiyagi

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village (GPS 0.472336, 32.802484) was randomly selected from Mukono district as the study area, and a quadrat random sampling technique was used to identify the occurrence of *S. anguivi* accessions. Fourteen *S. anguivi* accessions were available in the study area. Identification of the accessions was carried out with reference to the documented phenotypic characteristics of *S. anguivi*. (Bukenya and Carasco, 1995), while authentication was carried out by an expert taxonomist at the Department of Botany, Makerere University (Uganda). Samples were obtained from 12 plants per accession in May 2018. Branches from each accession were plucked, and the morphological characteristics were then assessed on the same day. Unripe fruits were collected from 12 plants per accession, pooled, and then dried to form flour as described under "sample preparation for chemical analysis". This entire process was replicated after two weeks to obtain second flour samples for each accession, and thus each accession had two flours collected independently.

Morphological characterisation

Morphological characterisation was based on the descriptors of tomatoes and eggplants (ECPGR, 2008), with modifications. Accessions in the present study were given codes (based on their morphological characteristics) for identification purposes. The accessions were characterized based on the amount and colour of pubescence on the leaves and stems and the colours of the leaves, stems, and fruits. Fruits were further characterized based on size/diameter (very small = < 0.8 cm, small = > 0.8 - 1.2 cm, intermediate = >1.2 - 1.8 cm, large = > 1.8 cm), shape, top (style scar area) appearance, venations, and also the average number of fruits per twig.

Sample preparation for chemical analysis

The fruits were sorted to remove those with damages on the pericarp. The stalks were then plucked off and the fruits were washed with distilled water and patted dry with a cotton cloth. Mature fruits (50) of similar sizes obtained from 12 plants for each accession were cut into four parts and those infested with pests were discarded. The sliced samples were dried in an oven [Infrared Food Oven GL-2A, Guangzhou Itop Kitchen Equipment Co, Ltd. Guangdong, China (Mainland)] at 40 °C for 16 hr. The samples were then milled (Wonder Mill, Pocatello, Idaho) at a "pastry" setting to obtain fine flour. The flours were stored in sealed plastic bottles at -20 °C until analysis. This entire process was replicated to get a second independent set of flours per accession from the fruits that were collected the second time.

Extraction and quantification of total bioactive compounds content and antioxidant activity of SALF

Extraction was carried out using 80% methanol (Kim et al., 2003) as described by Makkar (2003), with slight modifications. The dried and powdered fruits of 0.2 g was extracted in 20 ml 80% methanol three times for 10 min each time. The extracts were then pooled together for BCC and AA analyses. Each accession had two extracts from the two independent flour samples. The BCC and AA analyses were carried out in triplicates for each extract.

All absorbances were measured using an ultraviolet spectrophotometer (Perkin-Elmer 3100, Artisan Technology Group 101E Mercury Drive, Champaign, IL, USA) and 80% methanol was used as blank. All quantities were expressed on a dry weight basis (DWB). The total phenolic content (TPC) was measured using the Folin-Ciocalteu reagent (FCR) method (Singleton et al., 1998), estimated from a gallic acid (≥ 98%, Sigma-Aldrich, Germany) standard curve (0.1 mg stock solution, 0.02-1.0 µl) and expressed as gallic acid equivalent (GAE). Total flavonoids content (TFC) was determined as described by Kumar et al. (2012), estimated from a quercetin (≥ 95%, Sigma-Aldrich, Germany) standard curve (0.1mg stock solution, 0.02-1.0 µl), and expressed as quercetin equivalent (QE). Total saponin content (TSC) was determined using the method of Hiai et al. (1976), estimated from a diosgenin (≥ 93%, Sigma-Aldrich, Germany) standard curve (10 - 100 µg/ml) and expressed as diosgenin equivalent (DE). Vitamin C content was determined using the method of Omaye et al. (1979), estimated from an ascorbic acid (≥ 99.7%, Sigma-Aldrich, Germany) standard curve (0.02 mg/ml stock solution, 0.063 to 0.5 ml), and expressed as ascorbic acid equivalent (AAE). Total alkaloid content (TAL) was determined using the method by Harborne (1973) and computed as mg/g. The antioxidant activity (AA) was determined by free radical scavenging capacity (FRSC) and total antioxidant capacity (TAC). The sample extracts were allowed to react with the stable free radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (≥ 90%, Merck, Germany) assay according to Brand-Williams et al. (1995). Briefly, to 3.9 ml of 6×10^{-5} mol/l (24 mg/l) DPPH in methanol, 0.1 ml sample extract was added. The mixture was vortexed and kept in the dark for 30 min. The FRSC was calculated as:

$$\text{Free radical scavenging capacity (\%)} = ((\textit{Absorbance blank} - \textit{Absorbance of sample}) \times 100)/(\textit{Absorbance of blank}) \quad \text{Eq. (1)}$$

A quercetin standard curve was prepared with FRSC (%) against concentration (1-10 µg/ml), and the total antioxidant capacity (TAC) of the samples was subsequently estimated from the quercetin standard curve and expressed as QE.

HPLC quantification of phenolic compounds

HPLC quantification was carried out to assess the variation of phenolic compounds in SALF accessions. These included gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin, as reported by Elekofehinti et al. (2013b). HPLC analysis was carried out for all accessions except for GP1, which was excluded due to the small sample quantity. The fruit powders (0.5 g) were extracted in 5 ml methanol according to Elekofehinti et al. (2013b) for 30 min under constant shaking (IKA Vibrax VXR, IKA-Labortechnik, Staufen i.Br., Germany). The extracts were then filtered through Whatman filter papers (grade 597 ½, diameter 125 mm) (GE Healthcare-UK Limited, Chalfont St. Giles, UK), which were then dried (Speedvac Plus SC11A, Farmingdale, NY, USA). The dried filtrate was redissolved in 1 ml methanol, and filtered through a 0.45 µm syringe filter (Carl Roth). HPLC analysis was performed on an Agilent LC 1100-System as described by de Araújo et al. (2014) with slight modifications to reflect the instrument specifications and sample volume. An Eclipse Plus C18 column (4.6×250 mm, 5 µm) with matching guard was used with solvent A (10% methanol, 0.9% trifluoroacetic acid, TFA), solvent B (75% methanol, 0.25% TFA), and a flow rate of 0.5 mL/min. The gradient programme set-points were 15-30%B (11 min); 30-99%B (11 min, 8 min steady), 99-10%B (3 min), return to initial values (2 min); all relevant peaks were eluted between minutes 8 and 30. For UV detection, the wavelength was set to detect the different phenolic compounds in one run. Thus, 310 nm was used, except for the time of 8-12 min (280 nm) and from 24 onwards (250 nm). To quantify the phenolic and flavonoid compounds, 5 calibration curves (gallic acid, chlorogenic acid, caffeic acid, rutin, quercetin, with RT = 8.5, 17.4, 21.0, 25.2 and 28.2 min, respectively) with 7 concentrations (R^2>99.99) were applied. All samples were run in duplicate (injection volume 5 µL).

Statistical analysis

Statistical analyses were carried out using IBM SPSS for Windows Version 21 (Armonk, NY, USA). The BCC and AA data were proven for normality of distribution (Kolmogorov-Smirnov, and Shapiro-

Wilk). One-way ANOVA was carried out to determine statistically significant differences in sample means, and the *post-hoc* Duncan test was used to separate the means at $p < 0.05$. The results were expressed as mean ± standard error of the mean (SEM). No statistical analyses were carried out for the HPLC results. Pearson's correlation was carried out to determine linear relationships between AA and BCC. Equations for the prediction of TAC were derived using multilinear regression. A principal component analysis (PCA) was applied for BCC and AA, to determine the contribution of different variables (per component) to the variance of data. Categorisation of accessions based on chemical and morphological data was carried out using hierarchical (Ward's method) and two-step cluster analyses, respectively.

Results

Morphological characteristics

The morphological characteristics varied among the accessions (Tab. 1 and 2). The typical morphological features are shown in Fig. 1 and the morphological characteristics for leaves and stems of the fourteen accessions are summarised in Tab. 1. All accessions had green leaves (not shown) while 64.3% had green stems. The leaves in this study had sparse to dense pubescence, that was mostly purple (71.4%) in leaves, and white and dense (57.1%) in stems.

Half of the accessions had fruits with two colours (a combination of either light green, dark green, white, or cream) with each colour occupying half of the fruit from the scars (Tab. 2 and Fig. 2). The size of the fruits in the present study ranged from very small (< 0.8 cm) to large (> 1.8 cm), with none to intermediate venations. The fruits were mostly small (64.3%), spherical (92.7%), and flat at the top (style scar area) (64.3%). Dark or very dark green fruits had sparse parallel venation, while white or light greenish cream fruits had no venation. The twigs had an average of eight fruits.

Bioactive compounds content and antioxidant activities of SALF accessions

The BCC significantly differed among accessions (Tab. 3). The total phenolics (8.0-12.4 mg GAE/g DW) were highest in accession GP1 and least in WP1, while the total flavonoids (0.9-2.1 mg QE/g DW) were highest in GV2, GV3, and GP1 and least in CP1 and WP1. The total saponins (51.1-124.8 mg DE/g DW) were highest in GC2 and GV3, and least in GC5 and GV2. The total alkaloids (81.4-127.7 mg/g DW) were high and not significantly different among 10 accessions, with the least content in CP1 and GC1. Vitamin C (3.6-6.4 mg AAE/g DW) was highest in GV3 and GC2, and least in GC3 and LV1 accessions.

HPLC results showed that chlorogenic acid had the highest content of the analyzed phenolic compounds in most of the SALF accessions (Tab. 4). The HPLC results suggested variation of the phenolic compound contents amongst the SALF accessions.

The FRSC (3.4-11.5%) and TAC (1.2-4.6 mg QE/g DW) significantly differed among SALF accessions (Tab. 5). Accession GP1 had the highest FRSC and TAC, followed by GV1 while WP1 had the least.

Antioxidant activity and its relation with the content of the bioactive compounds

TAC and FRSC had similar statistically significant positive correlations with TPC (r = 0.74, $p = 0.000$), TFC (r = 0.23, $p = 0.038$) and TSC (r = 0.25, $p = 0.028$) (Tab. 6). Vitamin C had a statistically significant negative correlation with FRSC (r = -0.22, $p = 0.048$), while its negative correlation with TAC was not statistically significant.

Principal component analysis (PCA) grouped the data into three components which cumulatively explained 78.8% variation in the data (Fig. 3). Component 1 (FRSC, TAC and TPC), 2 (TSC and TAL) and 3 (TFC and vitamin C content) accounted for 39.8%, 21.9% and 17.1% variance in the data, respectively.

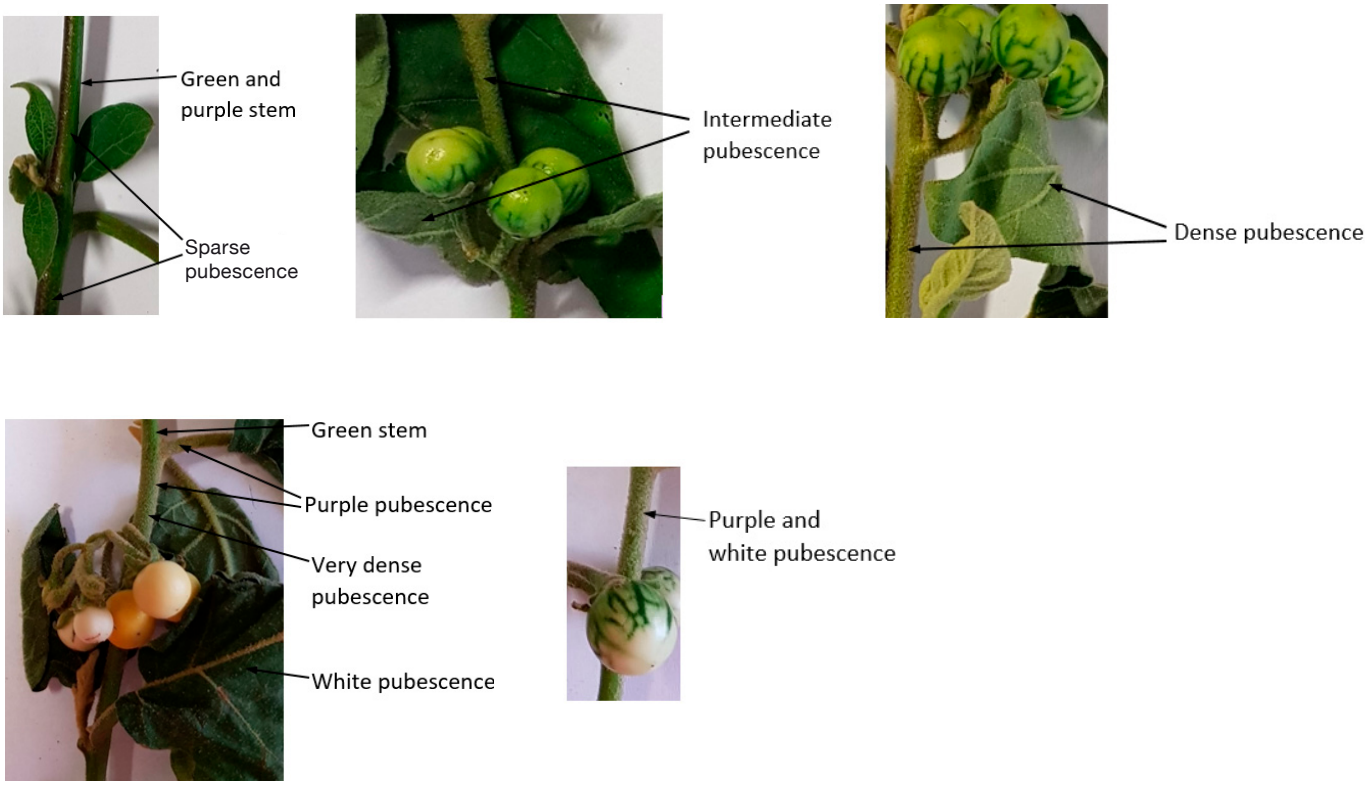

Fig. 1: Photographs of morphological characteristics of the leaves and stems of the *Solanum anguivi* Lam. accessions showing variation in pubescence density – sparse (A), intermediate (B), dense (C) and very dense (D); pubescence colour – purple (D), white (D) and purple and white (E); and stem colour – green (D), and green and purple (A).

Tab. 1: Morphological characterisation of leaves and stems of fourteen *Solanum anguivi* Lam. accessions.

Accession code	Leaf		Stem		
	Pub. distr.	Pub. colour	Pub. distr.	Pub. colour	Colour
LV1	Dense	White	Dense	White	Green and purple
GV1	Sparse	White	Sparse	White	Green and purple
GV4	Intermediate	Purple and white	Dense	Purple and white	Green
CP1	Intermediate	Purple and white	Dense	Purple and white	Green
GC1	Very dense	Purple and white	Very dense	Purple and white	Green
GC2	Dense	Purple and white	Dense	Purple and white	Green
GC3	Dense	Purple and white	Dense	Purple and white	Green and purple
GC4	Very dense	Purple and white	Very dense	Purple	Green and purple
GC5	Sparse	Purple and white	Intermediate	Purple and white	Green
WP1	Very dense	Purple and white	Very dense	Purple	Green
GV2	Intermediate	White	Dense	White	Green
GV3	Sparse	White	Sparse	White	Green and purple
GP1	Intermediate	Purple and white	Dense	Purple and white	Green
GC6	Dense	Purple and white	Dense	Purple and white	Green

Pub. distr. = Pubescence distribution, Pub. Colour = Pubescence colour

Tab. 2: Morphological characterisation of fruits of fourteen *Solanum anguivi* Lam. accessions.

Accession code	Colour	Size	Shape	Fruit top	Venation	Av. twig
LV1	Light green	Small	Spherical	Flat	Dense, reticulate	9
GV1	Very dark green	Very small	Spherical	Cuspidate	Sparse, parallel	8
GV4	Dark with light green	Very small	Spherical	Flat	Intermediate, reticulate	8
CP1	White	Small	Spherical	Flat	None	12
GC1	Light green with white	Small	Spherical	Cuspidate	Sparse, parallel	12
GC2	Dark green with white	Small	Spherical	Cuspidate	Sparse, parallel	12
GC3	Dark with light green	Small	Spherical	Flat	Intermediate, reticulate	8
GC4	Light green with white	Small	Spherical	Flat	Intermediate, reticulate	8
GC5	Dark green with cream	Intermediate	Ovoid	Elliptic	Intermediate, reticulate	8
WP1	White	Small	Spherical	Flat	None	5
GV2	Dark green	Large	Spherical	Flat	Sparse, parallel	1
GV3	Dark green	Small	Spherical	Flat	Sparse, parallel	6
GP1	Light greenish cream	Small	Spherical	Conical	None	8
GC6	Light green with white	Intermediate	Spherical	Flat	Intermediate, reticulate	10

Size= fruit size diameter (very small = < 0.8 cm, small = > 0.8 - 1.2 cm, intermediate = >1.2 - 1.8 cm, large = > 1.8 cm); Av. twig = average number of fruits per twig; fruit top = style scar area.

Regression models were derived to determine the BCC that affected SALF AA the most. Model 1 was for TAC mg QE/g DW (Eq. 2), and model 2 for FRSC (%) (Eq. 3).

I) Model 1

$$\text{TAC (mg QE/g)DW} = -3.087 + 0.661[TPC(\textit{mg GAE/g DW})] - 0.230[\textit{Vitamin C (mg A AE/g DW)} \quad \text{Eq. (2)}$$

Where, r = 0.784, $r^2 = 0.615$, adjusted $r^2 = 0.600$ and the unstandardized coefficients of the constant, phenolics and vitamin C were statistically significant at $p < .001$, .001 and .005 respectively.

II) Model 2

$$\text{FRSC \%} = -6.356 + 1.561[TPC(\textit{mg GAE/g DW})] - 0.608[\textit{Vitamin C (mg A AE/g DW)}] \quad \text{Eq. (3)}$$

Where r = 0.788, $r^2 = 0.621$, adjusted $r^2 = 0.607$ and the unstandardized coefficients of the constant, phenolics and vitamin C were statistically significant at $p < .005$, .001 and .005 respectively.

The regression models showed that total phenolic and vitamin C contents mostly affected the TAC and FRSC of SALF. The models (Eq. 2 and Eq. 3) derived in this study were good as indicated by their moderately high adjusted r^2. The models may thus explain 60% variance of the SALF AA.

Cluster analysis

Cluster analysis based on morphological characteristics revealed that leaf and stem pubescence colours and fruit colour were the most important for the categorisation of *S. anguivi* accessions (Fig. 4).

Cluster analysis based on the chemical compositions categorized the *S. anguivi* accessions into four clusters (Fig. 5). Cluster 1 accessions had low contents of TFC and TPC and TAC, with moderate to low vitamin C and TSC. Cluster 2 accessions had high TFC, TPC, TSC, and TAL, with low vitamin C. Cluster 3 accessions had low TPC, TSC, TAL, vitamin C, and TAC; with moderate TFC. Cluster 4 accessions had the highest TFC, TSC, TAL, and vitamin C; moderate levels of TPC, and the lowest TAC.

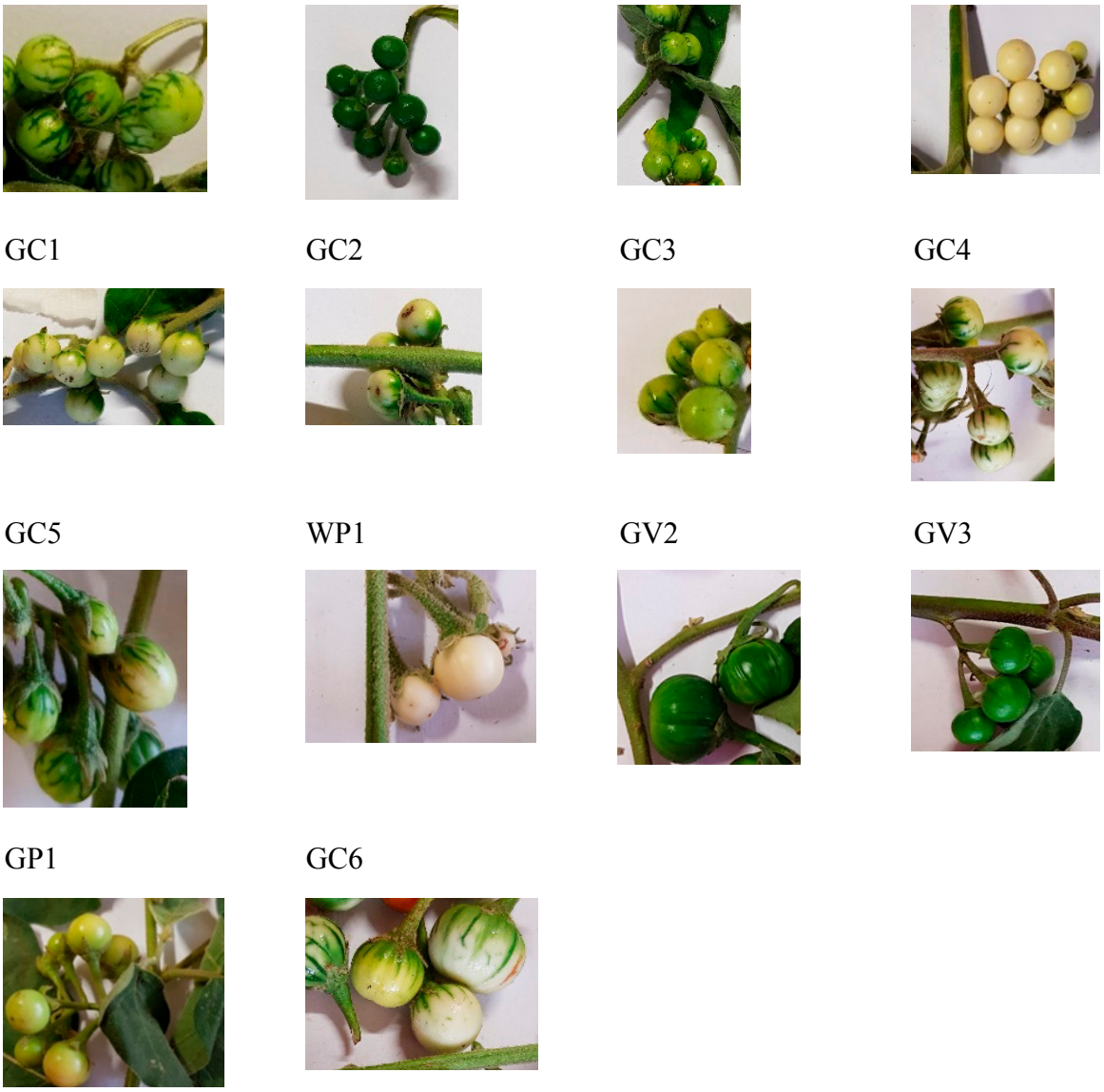

Fig. 2: Photographs of fourteen accessions of *Solanum anguivi* Lam. fruits. Photographs are not to scale. The accessions with one colour included LV1 (small, light green), GV1 (very small, very dark green), CP1 (small and white), WP1 (small, white), GV2 (large, dark green), and GV3 (small, dark green). Accessions with two colours included GV4 (very small, dark with light green), GC1 (small, light green with white), GC2 (small, dark green with white), GC3 (small, dark with light green), GC4 (small, light green with white), GC5 (intermediate size, dark green with cream), GP1 (small, light greenish cream) and GC6 (intermediate size, light green with white).

Comparison between cluster analysis based on morphological characteristics (Fig. 4) and that based on chemical compositions (Fig. 5) showed that 35.7% of the accessions, that is, 5 (GV4, GC3, CP1, GC4, and GC6) out of the 14, appeared together in both analyses.

Discussion

The morphological characteristics of *S. anguivi* plant accessions have been previously reported (Osei et al., 2010; Sseremba et al., 2017; Tembe et al., 2020); however, the data was limited. Osei et al. (2010) only reported that the SALF were small with venations, however, they did not define the small size and the venations of the SALF. Additionally, Sseremba et al. (2017) and Tembe et al. (2020) did not report the fruit colours and shapes and the leaf and stem pubescence distribution of SALF accessions. They only gave generalized characteristics of the *Solanum* species in their respective studies. Simultaneously, data on the appearance of the fruit top (style scar area), the average number of fruits per twig, and the amount and type of venations on the fruits are scarce and have been described in the present study (Tab. 2 and Fig. 2). Similar to our results, Osei et al. (2010) reported a round shape for SALF. Contrary to our findings (Tab. 1), Osei et al. (2010) observed an absence of pubescence on the leaves of *S. anguivi* accessions, and an absence to sparse pubescence in the leaves of *S. aethiopicum* and *S. macrocarpon* accessions. Furthermore, the SALF in the study by Osei et al. (2010) were small size, unlike the present study where the SALF accessions ranged from very small to large size (Tab. 2). The morphological variations in the present study may be due to genetic differences in the accessions. The analysis of molecular variance (AMOVA) by Stedje and Bukenya-Ziraba (2003) showed that the variance within *S. anguivi* accessions based on DNA data was great (90.42%). Therefore, the present study adds further information to the knowledge regarding morphological variations of *S. anguivi* accessions.

The differences in TPC among the accessions in the current study

Tab. 3: Bioactive compounds content of *Solanum anguivi* Lam. fruit accessions.

Accession code	TPC (mg GAE/g)	TFC (mg QE/g)	TSC (mg DE/g)	TAL (mg/g)	Vitamin C (mg AAE/g)
LV1	10.30±0.32 ^{c}	1.58±0.05 ^{b,c}	101.17±1.64 ^{c}	108.04±1.81 ^{a,b,c}	3.56±0.19 ^{g}
GV1	11.13±0.18 ^{b}	1.73±0.04 ^{b}	115.43±1.68 ^{b}	123.94±9.96 ^{a}	4.29±0.40 ^{f}
GV4	10.52±0.17 ^{b,c}	1.28±0.05 ^{c,d}	87.91±3.30 ^{e}	105.46±1.71 ^{a,b,c}	4.27±0.21 ^{f}
CP1	9.60±0.27 ^{d,e,f}	0.89±0.16 ^{e}	114.64±1.91 ^{b}	91.42±3.66 ^{c,d}	5.01±0.24 ^{c,d,e}
GC1	9.55±0.08 ^{d,e,f}	1.56±0.09 ^{b,c}	76.02±5.80 ^{f}	81.38±2.58 ^{d}	4.31±0.21 ^{f}
GC2	10.0±0.17 ^{c,d,e}	1.53±0.07 ^{b,c}	124.83±0.83 ^{a}	111.81±6.20 ^{a,b}	6.05±0.13 ^{a,b}
GC3	9.59±0.17 ^{d,e,f}	1.69±0.05 ^{b}	104.48±1.41 ^{c}	113.51±12.46 ^{a,b}	4.17±0.07 ^{f,g}
GC4	9.33±0.03 ^{e,f}	1.55±0.05 ^{b,c}	91.63±3.22 ^{d,e}	122.55±7.97 ^{a}	4.38±0.13 ^{e,f}
GC5	9.46±0.20 ^{d,e,f}	1.72±0.22 ^{b}	51.13±1.48 ^{g}	97.77±5.84 ^{b,c,d}	4.31±0.12 ^{f}
WP1	8.04±0.28 ^{g}	1.00±0.06 ^{d,e}	77.15±3.45 ^{f}	112.57±4.08 ^{a,b}	4.69±0.15 ^{d,e,f}
GV2	10.10±0.19 ^{c,d}	2.07±0.09 ^{a}	58.51±1.81 ^{g}	92.26±7.68 ^{c,d}	5.20±0.18 ^{c,d}
GV3	10.15±0.26 ^{c,d}	2.06±0.15 ^{a}	121.92±2.62 ^{a,b}	118.24±8.67 ^{a,b}	6.40±0.11 ^{a}

Solanum anguivi Lam. fruits were obtained from 12 plants per accession. The fruit batches were obtained twice (two weeks apart) to obtain two independent flour samples per accession. Values are the mean ± SEM of two independent experiments measured in triplicates. The means were computed on a dry weight basis. Means within the same column with different superscripts are significantly different at $p < 0.05$. TPC = total phenolic content, GAE = gallic acid equivalent, TFC= total flavonoid content, QE = quercetin equivalent, TSC = total saponin content, DE = diosgenin equivalent, TAL= total alkaloid content, AAE = ascorbic acid equivalent.

Tab. 4: HPLC quantification of phenolic compounds in *Solanum anguivi* Lam. fruit accessions.

Accession code	Gallic acid (µg/g)	Chlorogenic acid (µg/g)	Caffeic acid (µg/g)	Rutin (µg/g)	Quercetin (µg/g)
LV1	53.93	21.37	15.06	36.69	35.11
GV1	21.16	244.28	19.54	65.22	28.11
GV4	46.08	30.61	12.92	15.45	10.71
CP1	39.79	103.10	19.28	36.32	35.34
GC1	23.71	192.96	14.61	28.93	10.77
GC2	55.29	22.93	14.22	27.26	28.04
GC3	20.60	58.84	6.64	13.81	30.57
GC4	37.21	16.00	16.18	10.43	36.09
GC5	49.00	156.45	24.56	72.02	10.93
WP1	44.37	111.83	15.36	20.25	27.99
GV2	50.57	304.33	36.95	15.29	14.47
GV3	70.00	120.95	28.37	28.75	11.75
GC6	58.68	59.77	20.87	20.83	11.72

Solanum anguivi Lam. fruits were obtained from 12 plants per accession. Fruits for each accession were dried and one extract was obtained for each accession. Values are the means of duplicate analyses from one extract per accession. The means were computed on a dry weight basis. Statistical analysis was not carried out on the data.

may have been due to variations in the proportions of phenolic compounds contained in the different accessions as similarly reported for *S. melongena* genotypes by Stommel and Whitaker (2003). Chlorogenic acid content was the highest of the quantified phenolic compounds in the SALF accessions, and accessions with the highest chlorogenic acid had the highest TPC. The total flavonoid contents of the SALF accessions in this study may also have been due to variations in the contents of the flavonoid compounds such as rutin and quercetin. As observed in the TPC and TFC, the saponin and alkaloid compounds may have varied among the accessions and thus influenced the accessions' total saponin and alkaloid contents. Since the accessions in this study were obtained from the same location, the differences in the BCC may be attributed to differences in the genotypes of the accessions. In agreement with our findings, *S. melongena* accessions have been reported to significantly differ in TPC and vitamin C contents (Hanson et al., 2006), while eggplant cultivars have been reported to significantly differ in total flavonoids (Kaur et al., 2014), saponins and alkaloids (Agoreyo et al., 2012). To assess whether the contents of the bioactive compounds in SALF were substantial for potential health benefits, we compared the SALF BCC with foods that have been documented as rich sources. Plums and apricots have been documented as among the top 100 most rich polyphenol sources (Pérez-Jiménez et al., 2010). The TPC range of SALF accessions in this study was higher than for plums (5.6 mg GAE/g DW) (Miletić et al., 2014) and apricots (4.7 GAE/g DW) (Miletić et al., 2014), which suggested that all SALF accessions in this study were rich in phenolics. Flavonoids are reportedly rich in onions (Kozłowska and Szostak-Węgierek, 2018) with 1.3-2.1 mg QE/g DW (Sharma et al., 2014), similar to the values in the present study. The SALF accessions were also possibly rich in saponins and alkaloids due to their higher contents as compared to saponin-rich fenugreek genotypes (9.0-17.0 mg DE/g DW) (Arivalagan et al., 2013) and alkaloid-rich *S. torvum* (1.2 mg/g DW) (Pérez-Amador et al., 2007). The vitamin C content of the SALF accessions falls within the range for tomatoes (2.0-5.2 mg/g DW) (Hallmann, 2012), which are reported as rich sources (Lykkesfeldt et al., 2014). Therefore, all SALF accessions in the present study are potentially rich sources of total phenolics, flavonoids, saponins, alkaloids and vitamin C.

Tab. 5: Antioxidant activity of fruits from *Solanum anguivi* Lam. accessions.

Accession code	Total antioxidant capacity (mg QE/g DW)	Free radical scavenging capacity (%)
LV1	2.68±0.14 ^{c,d}	7.29±0.36 ^{c}
GV1	3.69±0.10 ^{b}	9.35±0.21 ^{b}
GV4	2.43±0.04 ^{d,e}	6.4±0.09 ^{d,e}
CP1	2.85±0.07 ^{c}	7.2±0.18 ^{c,d}
GC1	2.31±0.07 ^{e,f}	6.19±0.16 ^{e}
GC2	1.60±0.07 ^{h}	4.4±0.16 ^{g}
GC3	2.60±0.11 ^{c,d,e}	6.78±0.23 ^{c,d,e}
GC4	2.63±0.16 ^{c,d,e}	6.96±0.39 ^{c,d,e}
GC5	1.91±0.05 ^{g}	5.24±0.12 ^{f}
WP1	1.20±0.03 ^{i}	3.37±0.06 ^{h}
GV2	2.05±0.08 ^{f,g}	5.29±0.17 ^{f}
GV3	1.44±0.14 ^{h}	3.98±0.34 ^{g}
GP1	4.58±0.14 ^{a}	11.51±0.33 ^{a}
GC6	2.00±0.15 ^{g}	5.36±0.37 ^{f}

Solanum anguivi Lam. fruits were obtained from 12 plants per accession. The fruit batches were obtained twice, two weeks apart to obtain two independent flour samples per accession. Values are the mean ± SEM of two independent experiments measured in triplicates. The means were computed on a dry weight basis. Means within the same column with different superscripts are significantly different at $p < 0.05$. QE = quercetin equivalent, DW = dry weight.

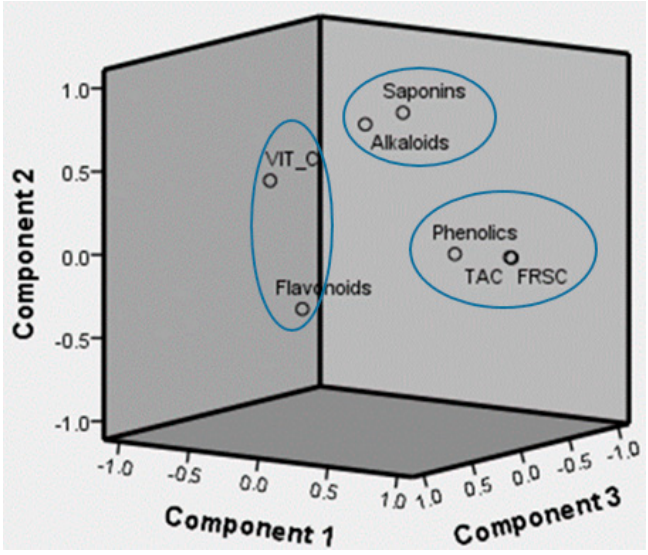

Fig. 3: Components derived from principal component analysis of *Solanum anguivi* Lam. fruits based on their bioactive compounds content and antioxidant activities. TAC = total antioxidant capacity, FRSC = free radical scavenging capacity, phenolics = total phenolic content, flavonoids = total flavonoid content, saponins = total saponin content, alkaloids = total alkaloid content, vit_C = vitamin C content. Component 1 = free radical scavenging capacity, total antioxidant capacity, and total phenolic content; component 2 = total saponin content and total alkaloids; and component 3 = Total flavonoid content and vitamin C content.

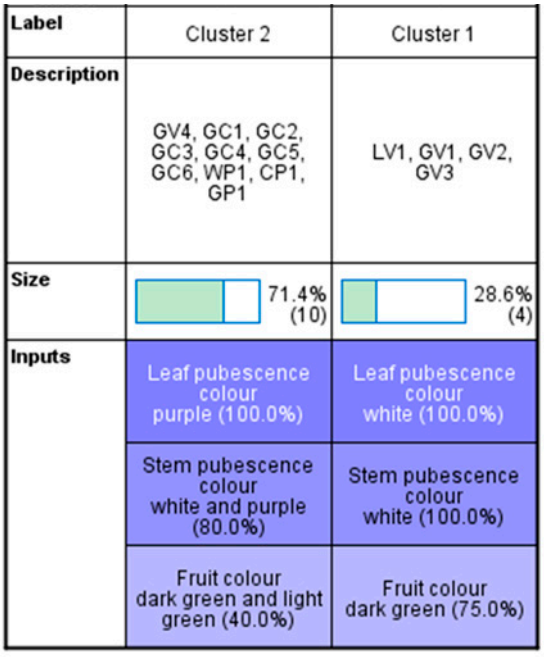

Fig. 4: Two-step cluster analysis of fourteen accessions of *Solanum anguivi* Lam. based on their morphological characteristics.

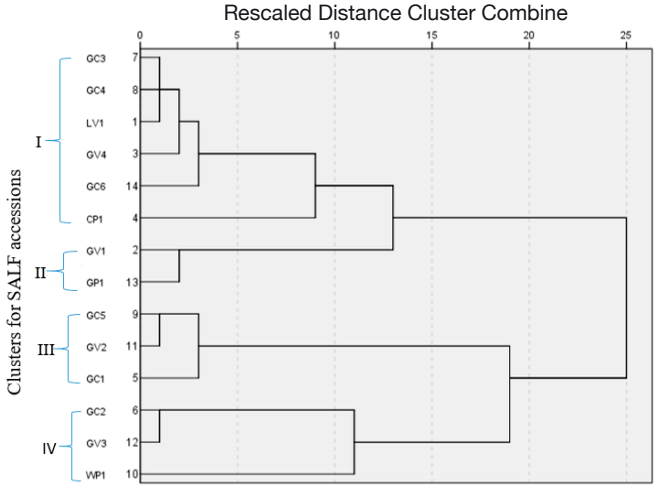

Fig. 5: A dendrogram representing diversity and clusters of fourteen *Solanum anguivi* Lam. fruit accessions based on their bioactive compounds content and total antioxidant capacity, using Ward's minimum variance in hierarchical cluster analysis. Cluster 1 accessions had low total flavonoids, phenolics and antioxidant activity, with moderate to low vitamin C and saponin contents. Cluster 2 accessions had high total flavonoids, phenolics, saponins and alkaloids, with low vitamin C content. Cluster 3 accessions had low total phenolics, saponins, alkaloids, vitamin C and antioxidant capacity, with moderate total flavonoid contents. Cluster 4 accessions had the highest total flavonoids, saponins, alkaloids and vitamin C, with moderate levels of total phenolics, and the lowest antioxidant capacity.

Free radicals are a major cause of the propagation stage of the oxidation process (Elekofehinti et al., 2013b) that may lead to oxidative stress. Bioactive compounds in fruits have been shown to suppress free-radical development, which further reduces the oxidative stress created in the body (Dhalaria et al., 2020). Consequently, the bioactive compounds protect the body against several diseases such as cancer, type 2 diabetes, inflammatory disorders, and other cardiovascular diseases (Dhalaria et al., 2020). In the present study, accession GP1 had the highest TAC and FRSC and may therefore possess the highest health benefits. Similar to the present study, Okmen et al. (2009) reported significant differences in the TAC of eggplant cultivars. The positive correlation between TSC and AA in this study

agrees with findings obtained by ELEKOFEHINTI et al. (2013a), who showed that SALF saponins had dose-dependent FRSC. Similar to our results, positive correlations between TPC and FRSC of *S. melongena* cultivars (OKMEN et al., 2009) and *S. melongena* accessions (HANSON et al., 2006) have been reported. The negative correlation between vitamin C and FRSC in the present study may show pro-oxidant properties of vitamin C in SALF. Similarly, PEYRAT-MAILLARD et al. (2001) reported that adding vitamin C to malt rootlet extracts led to an antagonist effect on their TAC. Vitamin C pro-oxidant properties are reported to be more evident under some circumstances, such as the presence of metal ions (iron and copper) and adequate pH (alkali) conditions (ARRIGONI and DE TULLIO, 2002). GHISLAINE et al. (2014) and OYEYEMI et al. (2015) reported the presence of iron (467.7 and 22.2 mg/100 g DW, respectively) and copper (0.1 and 1.4 mg/100 g DW, respectively) in SALF. Further studies investigating the antagonistic effect of vitamin C in SALF and its possible relationship with iron and copper contents may be suitable.

Cluster analysis was carried out to assess whether the morphological characteristics of *S. anguivi* may be used to predict the SALF accessions with similar BCC and AA. The clusters formed based on chemical compositions (Fig. 5) agree with the correlation results and the AA regression models in this study. Cluster 1 was characterized by low phenolics and flavonoids, which may explain their low TAC. Furthermore, the accessions that had moderate VCC and low saponin contents had the least TAC, and those with low VCC and moderate TSC had significantly higher TAC than the former. Cluster 2 accessions had chemical compositions similar to the correlation results obtained in this study: high phenolic, high flavonoid, high saponin, and low VCC, and high TAC. Although cluster 3 accessions were characterized by low vitamin C, and high flavonoid contents, the low phenolic and saponin contents may have resulted in the low TAC. The low TAC in cluster 4 may be due to the high VCC, whose negative effect may have suppressed the positive contribution on TAC by the moderate and high amounts of phenolics and saponins, respectively. BCC and TAC may therefore be good predictors for variation among SALF accessions given that the results from the cluster analysis were coherent with the correlation and regression analyses. Due to some similarities between the clusters formed based on morphological characteristics and based on chemical composition (35.7% of the accessions were grouped together in both cluster analyses), morphological features may be used to predict some accessions with similar chemical compositions.

Conclusion

The morphological characteristics of *S. anguivi* varied among the accessions. All accessions were rich in phenolics, flavonoids, saponins, alkaloids, and vitamin C. However, the BCC and AA significantly differed among the SALF accessions. Chlorogenic acid had the highest content of the analyzed phenolic compounds in the fruits. High amounts of total phenolic, flavonoid, or saponin contents may lead to high AA, while high vitamin C content may negatively affect the AA of SALF. Therefore, the results guide which accession one may consume to benefit from a given bioactive compound. The association between the morphological characteristics and the chemical compositions for some SALF accessions suggested that the morphological characteristics of *S. anguivi* Lam. may be used to predict accessions with similar BCC and AA.

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Conflict of interest

The authors reported no potential conflict of interest.

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