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Original Article

Rapid screening of toxic glycoalkaloids and micronutrients in edible nightshades (*Solanum* spp.)

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ABSTRACT

African indigenous vegetables (AIVs) because of their nutrient density have the unique potential to reduce micronutrient deficiencies in sub-Saharan Africa, yet some may also contain anti-nutritive compounds. Vegetable nightshades from *Solanum americanum*, *Solanum nigrum*, *Solanum scabrum* and *Solanum villosum* are among the major AIVs used as a leafy vegetables and consumed regularly in many countries in sub-Sahara Africa. These under-recognized food crops have not been subjected to extensive studies for their nutritional and antinutritive factors. In this study, 15 entries of the vegetable nightshades were field-grown and the leaves which are the consumed product of commerce chemically profiled by LC/ESI-MS. Twenty-three flavones, eight saponins, and two glycoalkaloids along with a phenolic acid of chlorogenic acid were identified by MS and UV data. Anti-nutrient glycoalkaloids were quantified as total aglycones after acidic hydrolysis using MS detection and found to be within safe-consumption thresholds by comparison with the glycoalkaloid level in the globally consumed *Solanum* member eggplants. Edible nightshades were also found to be sources of β-carotene, vitamin E and total polyphenols and exhibited high antioxidant activity. Results of this study support that consumption of vegetable nightshades are safe from the presence of glycoalkaloids and thus, can contribute to the reduction of micronutrient deficiency in sub-Sahara Africa.

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1. Introduction

African indigenous vegetables (AIVs) are consumed as important nutrient-rich foods locally and regionally in sub-Saharan Africa, with many also utilized for their medicinal properties [1]. Such AIVs, also called traditional African vegetables, are collected from the wild or cultivated to a limited extent and consumed or marketed, serving as an important income generating opportunity for the typical small-scale farmer, especially in such economically limited regions [2]. Adapted to the local environment, AIVs often provide more sustainable production than exotic or introduced crops such as European vegetables [3]. Efforts are being made to increase the farming and marketing of AIVs in an attempt to alleviate hunger and improve nutrition, and to increase farmers income, improving the local and regional economy [3].

African nightshades are among the most popular and as such high priority leafy AIVs, and their leaves as well as tender stems can be steamed and eaten like spinach and amaranth. The edible nightshades represent a wide group of botanically and genetically related plants belonging to approximately 30 species in the *Solanum* genus of the Solanaceae family, and are diversely referred to as vegetable nightshades, edible nightshades, garden nightshades, common nightshades, '*S. nigrum* complex', or '*S. nigrum*' and related species [4]. Despite their frequently reported nutritional attributes, *Solanum* species are also well known to contain toxic alkaloids, such as glycosides of solasodine and solanidine [5]. This safety concern is associated with the edible African *Solanum* nightshade species, as these compounds are known to be present in the fruits [6] and have limited the promotion of their cultivation and marketing.

As edible nightshades are consumed in sub-Sahara Africa for their leaves, not the fruits, the presence of glycoalkaloids in leaves could present health concerns. Therefore, the purpose of this research was to determine the nutritional content of edible African nightshades and to also examine whether glycoalkaloids are present in the leaves, and if so to identify and quantitate such compounds. The results would therefore clarify consumption safety concern and promote the cultivation and marketing of leafy AIVs, or identify if any sources are free and devoid of such compounds for breeding and crop improvement programs. In this investigation, 15 African edible nightshade entries were chemically profiled by LC/UV/MS to identify major bioactive compounds as well as toxic glycoalkaloids. Additionally, the other nutritive values were measured including β-carotene and vitamin E level, total polyphenol content (TPP) and total antioxidant activity.

2. Materials and methods

2.1. Chemical reagents

Standard compounds solasodine was purchased from MP Biomedicals (Santa Ana, CA, USA) and solamargine from MedChem Express (Monmouth Junction, NJ, USA). Standards β-carotene and vitamin E (α-tocopherol), Folin Ciocalteu's phenol reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) from Sigma–Aldrich (St. Louis, MO, USA). Gallic acid was purchased from Acros Organics (Belgium, WI, USA) and acetone from BDH Chemicals (Radnor, PA, USA). Methanol, ethyl acetate, *tert*-butyl methyl ether, concentrated hydrochloric acid, and HPLC grade water and acetonitrile modified with 0.1% formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Equipment

A propane-heated walk-in Powell Maxi Miser tobacco dryer (Bennettsville, SC) was used for sample drying. Agilent 1100 series LC/MSD instrument (Waldbronn, Germany) was used for phytochemical profiling. The HPLC was equipped with an auto-degasser, quaternary pump, thermostatted column compartment and a diode-array detector (DAD). Column Agilent Polaris 3 Amide C18, 250 × 4.6 mm (Santa Clara, CA, USA) was used for phytochemical profiling, and column Phenomenex Prodigy ODS-3150 × 4.6 mm, 5 μm (Torrance, CA, USA) was used for quantification of total glycoalkaloids. The HPLC-MS interface used an electrospray ionization source (ESI) and the MS featured an ion trap analyzer. The software used was HP ChemStation, Bruker Daltonics 4.1 and DataAnalysis 4.1. Waters 2695 HPLC (Milford, MA, USA) was used for β-carotene and vitamin E measurement, which was equipped with a quaternary pump and a DAD. The separation was achieved by YMC-C30 carotenoid column, 5 μm, 250 × 4.6 mm (YMC Co., Ltd). The software was Millennium 4.00. Bio-Tek Synergy HT Multi-Mode Microplate reader (Winooski, VT, USA) was used for spectrophotometric measurement for total polyphenol assay and antioxidant assay. The software used was Bio-Tek KC4 Version 3.4.

2.3. Plant samples

Seeds of 15 entries of *S. nigrum*, *Solanum scabrum*, *Solanum americanum* and *Solanum villosum* (Table 3) were sown under greenhouse conditions at the Rutgers Research Greenhouses in New Brunswick, NJ. After four weeks of growth, the seedlings were transplanted during the first week of June in 2015 into a cultivated field at the Clifford E. & Melda C. Snyder Research and Extension Farm, New Jersey Agricultural Experiment Station of Rutgers University in Pittstown, New Jersey (40.6°N, 75.0°W, 116 m elevation). The leaves of the nightshades were manually harvested with the first harvest occurring 21–28 days post-field transplanting. The aerial parts were cut ~15 cm above the soil line to allow the plants to regrow for multiple harvesting.

2.4. Sample preparation

The collected aerial parts were dried at 40 C° for two weeks and then ground into powder. The samples were stored in shaded zip-lock bags under room temperature. For phytochemical profiling by LC/UV/MS, total polyphenol (TPP) assay and antioxidant assay, around 200 mg of the sample was accurately weighed and extracted with 25 mL 70% methanol with 0.1% formic acid. Each extract was fully vortexed, sonicated in a water bath for 5 min, and then let stand still

Table 1 – Peak assignments used for the analysis of 15 edible nightshade (*Solanum* spp.) accessions.

Peak/Compound code	RT(min)	Compound ID	$[M+H]^+/[M+Na]^+$ (m/z)	Fragment ion (m/z)
1	13.6	Chlorogenic acid	377, 355[a]	–
2	14.0	Quercetin-G-Rha-G	795[a]	611, 465, 303
3	16.2	Quercetin-G-Xyl-Xyl	751[a]	597, 465, 303
4	16.8	Quercetin-G-Rha-Rha	779[a]	611, 465, 303
5	17.8	Quercetin-G-G	649[a]	465, 303
6	18.1	Quercetin-G-Rha-Xyl	765[a]	465, 303
7	18.3	Solasodine-G-G-Rha	884	576, 414
8	18.7	Quercetin-G-Xyl	619[a]	465, 303
9	18.9	Quercetin-G-Xyl	619[a]	465, 303
10	19.1	Kaempferol-G-G	633[a]	449, 287
11	20.0	Solasodine-G-Rha-Rha	868	722, 576, 414
12	20.2	Kaempferol-G-Xyl	603[a]	449, 287
13	20.6	Kaempferol-G-Xyl	603[a]	287
14	20.7	Kaempferol-G-G	633[a]	449, 287
15	21.4	Rhamnetin-G-Xyl-Xyl	765[a]	611, 479, 317
16	21.9	Quercetin-G-Rha	633[a]	465, 303
17	24.0	Quercetin-G	487[a]	303
18	24.0	Rhamnetin-G-G	663[a]	479, 317
19	25.3	Rhamnetin-G-Xyl	633[a]	479, 317
20	25.5	Kaempferol-G-Xyl	617[a]	449, 287
21	25.6	Rhamnetin-G-Xyl	633[a]	479, 317
22	26.9	Rhamnetin-G-Xyl	633[a]	479, 317
23	27.4	Kaempferide-G-Xyl	617[a]	301
24	27.8	Kaempferide-G-Xyl	617[a]	463, 301
25	28.6	Rhamnetin-G-Xyl	633[a]	479, 317
26	29.5	Rhamnetin-G-Rha	647[a]	479, 317
27	33.3	Tigogenin-G-G-G-G-G	1249[a]	1087, 903, 741, 579, 417
28	34.4	Tigogenin-G-G-G-Xyl-G	1197	1057, 903, 741, 579, 417[a]
29	34.8	Dehydrodiosgenin-G-G-Rha-Rha	1029	883, 737, 575, 413
30	35.7	Diosgenin-G-G-Rha-Rha	1031	885, 739, 577, 415
31	41.5	Tigogenin-G-G-Rha-Xyl-Xyl	1173[a]	887, 741, 579, 417
32	42.6	Tigogenin-G-G-Rha-Xyl-Xyl	1173[a]	887, 741, 579, 417
33	43.1	Dihydromethyldiosgein-G-Rha-Rha-G	1069[a]	885, 739, 593, 431
34	45.6	Tigogenin-G-G-G	903	741, 579, 417

[a] Sodium adducted ions; RT, retention time; G, glucosyl, galactosyl, mannosyl or other hexosyl; Rha, rhamnosyl; Xyl, Xylosyl.

overnight under room temperature [7–9]. The extract was centrifuged at 3000 rpm/min for 10 min. For TPP assay, the supernatant was directly ready for analysis. For antioxidant activity assessment, the supernatant was diluted twice by 70% methanol with 0.1% formic acid before the assay. For phytochemical screening by LC/ESI-MS, the supernatant was filtered by 0.45 μm filter prior to HPLC injection.

For quantification of total alkaloid aglycone, the sample was prepared based on protocols by P.G. Crabbe [10] with modification. Briefly, around 400 mg of sample was accurately weighed and hydrolyzed by 20 mL 0.5 M anhydrous methanolic hydrochloric acid in an eight dram vial with a screw cap, and incubated in a 70 °C water bath for 120 min. After incubation, the hydrolyte was cooled down and basified with 3 mL saturated sodium hydroxide methanolic solution to terminate the hydrolysis reaction, and was then brought to 25 mL by methanol. The final hydrolyte was filtered by 0.45 μm filter before LC/MS injection. For construction of the calibration curve and determination of limit of quantification (LOQ) and limit of detection (LOD), 4.7 mg solasodine standard was dissolved in 10 mL methanol as stock solution, and diluted 100 times as the first work solution, followed by successive two-fold dilution down to sub-nanogram/mL. LOQ was defined as the signal to noise ratio (S/N) at 10:1, and LOD as S/N at 3:1. The recovery rate was evaluated by spiking known amount of authentic standard solamargine to approximately 200%, 100% and 50% of the expected values in the sample in terms of total aglycone and then hydrolyzing following the same procedure.

The aforementioned hydrolysis method was also applied to release aglycones of flavonoids and saponins for further confirmation of their structures.

For the analysis of β-carotene and vitamin E, about 500 mg sample was weighted accurately and extracted by 5 mL acetone in a two dram vial, fully vortexed and sonicated for 30 s. The extract was then centrifuged at 3000 rpm for 5 min with the supernatant decanted to an 8 dram amber vial. The sample was subsequently extracted in like manner by another 5 mL acetone and 2 mL *tert*-butyl methyl ether, respectively, with the supernatants combined in the 8 dram vial. The supernatant was then rotovapped to yield the dry extract. The dried residue was then reconstituted into 2 mL 1:1 ethyl acetate: methanol, followed by centrifugation at 3000 rpm for 10 min, and the supernatant was ready for HPLC analysis. The extract for each entry was prepared in triplicate.

Table 2 – Phytochemical profile analyses of 15 edible nightshades (*Solanum* spp.) accessions.

Peak no.	S.n 1	S.n 2	S.n 3	S.n 4	S.n 5	S.s 1	S.s 2	S.s 3	S.s 4	S.s 5	S.s 6	S.s 7	S.s 8	S.a 1	S.v 1
1	–	T	+	T	T	+	–	+	+	+	+	T	T	+	T
2	–	–	–	–	+	+	–	+	+	T		–	–	++	++
3	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	+	T	++	+	++	+	+	++	+	+		–
5	++	++	+	T	++	+	T	+	+	+	T	T	T	++	++
6	–	–	–	–	–	–	T	+	+	+	T	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T
8	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
9	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–
10	+	+	–	–	+	–	–	–	–	–	–	–	–	T	–
11	–	–	–	–	T	–	–	–	–	–	–	–	–	–	T
12	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–
13	–	–	T	–	–	–	–	–	–	–	–	–	–	T	–
14	–	–	–	–	–	–	–	T	–	T	–	–	–	–	–
15	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–
16	T	–	+	+	T	+	+	++	+	+	++	+	+	–	T
17	+	T	+	–	+	–	–	–	–	–	–	–	–	T	
18	–	–	+	–	T	–	–	–	–	–	–	–	–	++	+
19	–	–	++	–		–	–	–	–	–	–	–	–	–	–
20	–	–	–	T		T	–	T	T	T	T				–
21	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–
22	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–
23	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–
24	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–
25	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–
26	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
27	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
28	++	++	–	++	++	++	++	++	++	++	++	++	++	++	++
29	–	–	++	–	–	–	–	–	–	–		–	–	–	–
30	–	–	++	–	–	–	–	–	–	–		–	–	–	–
31	+	++	–	+	++	++	+	++	++	++	+	+	+	++	+
32	+	+	–	+	++	++	+	++	++	++	+	+	+	+	+
33	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–
34	+	++	–	–	–	–	–	–	–	–	–	–	–	–	+

Compound codes refer to Table 1 and the plant sample code are the same as in Table 3. "++", strong peaks defined as intensity of UV–vis over 10 mAU or of extracted ion chromatograph (EIC) by extraction of molecular ions and corresponding fragment ions over 5×10^5; "T", trace level, defined as intensity of UV–vis lower than 1 mAU or EIC intensity lower than 5×10^4; "+", peak intensity between "++" and "T"; "–", peaks not detected.

2.5. *Phytochemical profiling*

The aerial part extract was chemically profiled by HPLC/UV/ESI-MS. HPLC grade water with 0.1% formic acid was used for mobile phase A and acetonitrile with 0.1% formic acid for mobile phase B. The gradient started from 10% to 20% B in 0–10 min, 20% to 28% in 10–30 min, 28%–30% in 30–40 min, and 30% to 50% in 40–60 min, followed by 5 min column flushing with 80% B and another 5 min column equilibration with the starting mobile phase before the next injection. The flow rate was 1 mL/min and the injection volume was 10 μl. The column was kept at 25 °C. The DAD detector was set at 210 nm, 254 nm, 280 nm and 370 nm for signals, and scanning range 200–550 nm with 2.0 nm step for spectrum. About a third of the HPLC eluent was split into the MS detector. In the ESI, the nebulizer needle voltage was set at 3500 V of positive polarity. High purity nitrogen was used as both nebulizing gas at 40 psi and drying gas at 350 °C with a flow rate of 10 L/min. High purity helium was used as the collision gas and the collision energy was set at arbitrary compound stability 80%. The scanning mode was set at positive and the range at 200–1300 *m/z*.

2.6. *Quantification of total glycoalkaloids in hydrolyzed nightshade samples using LC/MS*

For MS detection, selected ion monitoring (SIM) mode was used for the selected target of solasodine at 414.7 *m/z* with a band width of 1.0 *m/z*, and the HPLC gradient started from 20% to 40% in 10 min, followed by 5 min column flushing with 80% B. The column was equilibrated by the starting mobile phase for 5 min before each injection. The other HPLC/MS parameters remained the same as phytochemical profiling. The aglycone content was reported as μg/g dry weight (DW).

2.7. *β-carotene and vitamin E analysis*

The β-carotene and vitamin E content was analyzed using Waters HPLC. Mobile phase A was micron-filtered 98:2 methanol:1 M ammonium acetate, and mobile phase B was HPLC grade ethyl acetate, and both were manually degassed by sonication under vacuum. The gradient started from 0% to 35% B in 0–8 min, 35% to 40% in 8–28 min, 40% to 50% in 28–32 min, and 50%–60% in 32–36 min and held until 40 min. The injection volume was 10 μl. UV chromatograms for β-

Table 3 – Identification, alkaloid aglycone and nutrients level from 15 accessions of edible nightshades (*Solanum* spp.).

Sample code[a]	Source[b]	Source ID	Glycoalkaloid aglycone (μg/g DW)	Vitamin E (μg/g DW)	β-carotene (μg/g DW)	ABTS (TE mg/g DW)	TPP (GAE mg/g DW)
S.n 1	Simlaw Seeds (Kenya)	–	ND	106.9 ± 2.0^{def}	78.4 ± 4.5^{bcd}	16.80 ± 0.64^{gh}	8.54 ± 0.33^{gef}
S.n 2	USDA	PI 306400	ND	92.0 ± 2.6^{gf}	28.1 ± 1.1^{f}	15.49 ± 0.69^{h}	7.25 ± 0.36^{h}
S.n 3	USDA	PI 312110	ND	229.7 ± 19.9^{a}	141.7 ± 11.7^{a}	23.45 ± 0.68^{abc}	11.92 ± 0.49^{ab}
S.n 4	USDA	PI 381289	ND	125.9 ± 7.6^{def}	70.6 ± 2.5^{ced}	18.34 ± 0.77^{gf}	9.58 ± 0.33^{de}
S.n 5	USDA	PI 381290	7.1 ± 2.3^{a}	197.1 ± 17.9^{ab}	86.7 ± 8.9^{bc}	23.93 ± 1.12^{ab}	13.07 ± 0.42^{a}
S.s 1	WorldVeg	SS 52	ND	114.9 ± 8.6^{def}	87.4 ± 3.7^{bc}	21.36 ± 0.50^{dc}	10.8 ± 0.35^{bc}
S.s 2	WorldVeg	Ex Hai	ND	90.4 ± 3.7^{gf}	64.8 ± 0.3^{ed}	17.92 ± 0.45^{gf}	7.98 ± 0.26^{ghf}
S.s 3	WorldVeg	SS 49; Olevolosi	ND	141.3 ± 1.9^{de}	96.0 ± 2.8^{b}	22.46 ± 1.11^{bdc}	12.21 ± 0.53^{a}
S.s 4	WorldVeg	SS 04.2	ND	64.1 ± 2.8^{g}	65.2 ± 2.0^{ed}	18.11 ± 0.62^{gf}	9.39 ± 0.28^{de}
S.s 5	WorldVeg	BG 16; Nduruma	ND	192.5 ± 28.5^{ab}	91.5 ± 13.0^{bc}	25.00 ± 0.70^{a}	12.47 ± 0.42^{a}
S.s 6	WorldVeg	BG-29	ND	102.1 ± 11.7^{gef}	55.1 ± 7.3^{e}	19.14 ± 0.33^{ef}	9.13 ± 0.24^{def}
S.s 7	USDA	Grif 14198	ND	121.1 ± 8.6^{def}	87.7 ± 4.7^{bc}	21.26 ± 0.59^{d}	10.98 ± 0.40^{bc}
S.s 8	USDA	PI 643126	ND	183.4 ± 30.1^{bc}	87.2 ± 14.0^{bc}	16.22 ± 0.33^{gh}	7.75 ± 0.52^{gh}
S.a 1	USDA	PI 268152	ND	145.5 ± 5.2^{dc}	95.8 ± 3.8^{b}	24.81 ± 0.09^{a}	12.44 ± 0.43^{a}
S.v 1	USDA	Grif 16939	38.0 ± 11.4^{b}	114.3 ± 5.0^{def}	138.1 ± 4.0^{a}	20.99 ± 0.99^{ed}	10.26 ± 0.40^{dc}

The results were reported as mean ± STD (n = 3). [a–h], values with same letters in the same column are not significantly different (ANOVA with Tukey's HSD test, $p < 0.05$).

[a] S.n: *Solanum nigrum*; S.s: *S. scabrum*; S.a: *S. americanum*; S.v: *S. villosum*. ND, not detected.

[b] Seeds from USDA were maintained by Plant Genetic Resources Conservation Unit, Griffin, GA. Seeds from World Vegetable Center (WorldVeg) were maintained by the regional center of east and southern Africa, Arusha, Tanzania. Italicized values of β-carotene contents were identified to be a source of vitamin A based on retinol equivalent (RE), assuming 90% moisture content in fresh vegetables [24,25].

carotene and vitamin E were generated at their maximum peak absorption wavelength of 452 nm and 295 nm, respectively, and both compounds were identified in comparison with their retention time and UV–Vis spectrum of the authenticated standards. For the calibration curve, around 20 mg vitamin E and 10 mg β-carotene standards were accurately weighed and dissolved in 25 mL ethyl acetate as stock solutions, respectively [11]. The stock solutions were then diluted by ethyl acetate in series to generate work solutions of ~4–~150 μg/mL for both compounds. The β-carotene and vitamin E content in samples was reported as μg/g DW.

2.8. Total polyphenol (TPP)

The method of TPP assay was based on that proposed by Singleton [12] and Kao et al. [13]with modification. First, 50 mL Folin Ciocalteu's reagent was diluted by distilled water to 500 mL, then 900 μL Folin reagent was mixed with 80 μL sample extract followed by the addition of 400 μL saturated sodium carbonate solution. The reaction system was fully vortexed and let stand still for 1 h. Then 200 μL supernatant was transferred to a 96 multiple well and subject to absorption measurement under 765 nm. As to the calibration curve, 20.0 mg gallic acid was dissolved in 5 mL 70% methanol as the stock solution, and diluted to a series of work solutions with concentrations ranging from 15.6 μg/mL to 250 μg/mL 200 μL 70% methanol was used as negative control. The assay for each calibrator and sample was triplicated. Total polyphenol content in samples was expressed as the amount of gallic acid equivalent (GAE)/g DW.

2.9. Antioxidant activity

The antioxidant activity assay was based on the method proposed by Re et al. [14] and Nagulsamy [15] with modification. First, 31.7 mg ABTS and 8.6 mg potassium persulfate were dissolved in 10 mL water and let stand still in darkness under room temperature for 12–16 h to form stable radical, and diluted to an absorption of ~1.3 at 734 nm. Next, 200 μl ABTS was mixed with 20 μl sample extract and let stand still for 15 min under room temperature, and the absorption was measured at 734 nm. For the calibration curve, 12.5 mg standard Trolox was dissolved in 5 mL pure ethanol as the stock solution, and diluted to series of work solutions with concentrations ranging from 19.5 μg/mL to 195.3 μg/mL. The assay for each calibrator and sample was conducted in triplicate. Antioxidant activity was expressed as the amount of Trolox equivalent (TE)/g DW.

2.10. Statistics

Data were represented in the form of mean ± the standard deviation (STD) of three replicates of each sample. Data was analyzed using student's *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test ($p < 0.05$). The relationship between TPP content and antioxidant activity was evaluated by Pearson's correlation. All statistics was performed using SAS University Edition (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Phytochemical profiling

Major peaks were identified based on the retention time, UV–Vis spectrum, and MS spectral data, as well as by comparison with authentic standards. The identities, retention time and MS data of individual compounds were summarized in Table 1. In the four *Solanum* species, flavonoids and

saponins dominate the secondary metabolites portfolio along with phenolic acid chlorogenic acid, occasionally with trace level of glycoalkaloids. Apparent variance in chemical profile was observed within species, as well as striking similarities between them, as commonly observed in the *Solanum* complex [16]. The chemical profile of 15 different accessions was summarized in Table 2.

3.1.1. *Flavonoids and phenolic acid*

Flavonoids were previously reported as an important class of bioactive compounds in nightshades [16]. In this work, a major phenolic acid chlorogenic acid along with an abundance of flavonols were detected and identified in the four AIVs, including glycosylated derivatives of quercetin, kaempferol, rhamnetin and kaempferide. Simultaneous UV and MS chromatograms of a representative nightshade extract were illustrated in Fig. 1. The individual peaks were identified based on UV–Vis spectrum and MS data. For the major phenolic acid, compound **1** (RT 13.6 min) exhibited adduct molecular ions at 377 ($[M+Na]^+$) and 355 *m/z* ($[M+H]^+$) and maximum UV peaks at 208 nm, 245 nm and 328 nm, suggesting a chlorogenic acid, neochlorogenic acid or cryptochlorogenic acid which differ at the esterification site of the quinoyl [17]. This compound was further confirmed to be chlorogenic acid by comparison with authentic standards. For identification of flavonols, for example, compound **8** (RT 18.7 min) featured a sodium adduct molecular ion at 619 *m/z* ($[M+Na]^+$), which was fragmented into 465 *m/z* ([M-xylosyl + H]$^+$), followed by aglycone ion at 303 *m/z* ([M-xylosyl-hexosyl + H]$^+$) which corresponded to the molecular mass of protonated quercetin. The maximal UV–vis absorption was around 360 nm, in agreement with that of quercetin glycosides in literature [17]. Thus, compound **8** was identified as quercetin conjugated with hexosyl and xylosyl. The above identification principle also applied to the other flavonol glycosides. In addition, the identification of aglycones of quercetin, kaempferol and rhamnetin were further confirmed by comparison with retention time of authentic standards after acid-assisted hydrolysis.

3.1.2. *Glycoalkaloids*

Glycoalkaloids were previously reported in leaves of all four *Solanum* species, though sometimes in disagreement. *S. nigrum* was extensively reported to contain solasodine glycosides including solamargine and solasonine and those with varied oligosaccharide chain [18,19]. Solanine, a glycoside of solanidine, was only occasionally reported at a content of 0.043 mg/g [20]. In *S. scabrum*, solamargine and solasonine was reported to be present [21], while the existence of alkaloids was decided to be "doubtful" in another study [22]. In a recent research, a screening by LC-QToF-MS revealed a lack of glycoalkaloids in the methanol extracts of both *S. scabrum* and *S. villosum* [23]. *S. americanum* was reported to contain glycosides of solasodine [24].

In this study, no alkaloids were detected in the leaves of the plants except in two accessions, *S. villosum* Grif 16939 and *S. nigrum* PI 381290, where two glycosides of solasodine was found (Fig. 2A). Compound **11** (RT 20.0 min), for example, has a molecular ion peak at 868 *m/z* $[M+H]^+$, followed by fragment ion 722 *m/z* [M-rhamnosyl + H]$^+$ and 576 *m/z* [M-rhamnosyl-

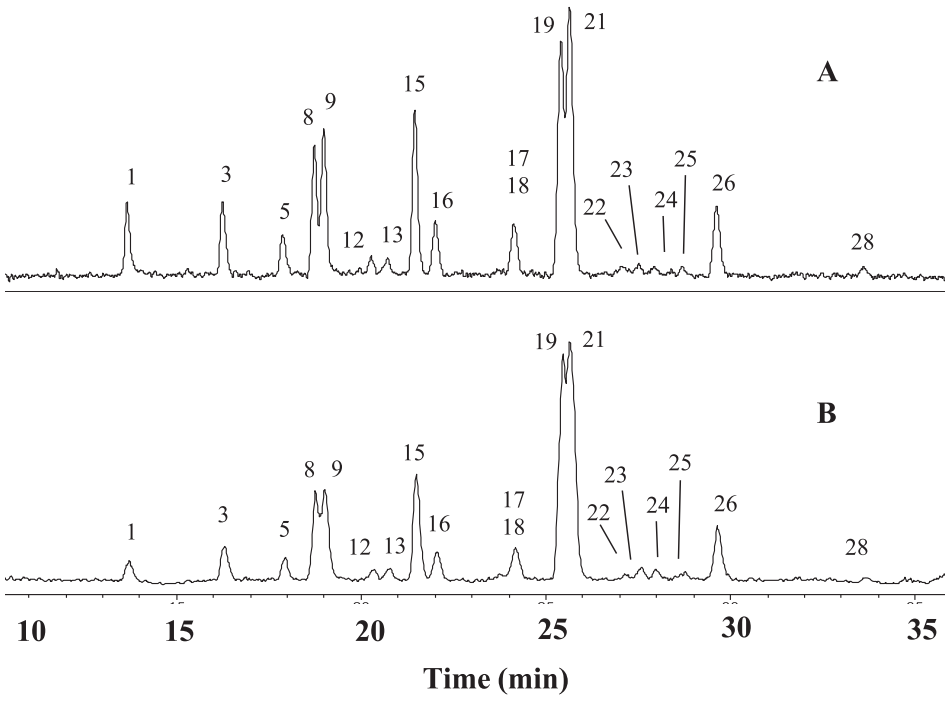

Fig. 1 – Representative HPLC/UV/MS chromatograms of *Solanum nigrum* PI 312110. (A) UV–vis chromatogram at 370 nm. (B) Processed MS chromatogram extracting molecular ions and characteristic fragment ions. The identities, retention time and MS of each peak are listed in Table 1.

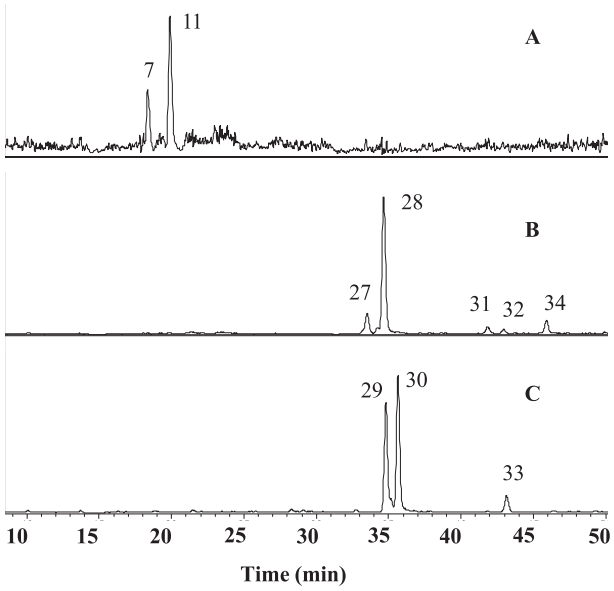

Fig. 2 – Processed MS chromatograms showing glycoalkaloids in *Solanum villosum* Grif 16939 (A) and saponins in *Solanum villosum* Grif 16939 (B) and *Solanum nigrum* PI 312110 (C).

rhamnosyl + H]$^+$, and finally 414 *m/z* [M-rhamnosyl-rhamnosyl-hexosyl + H]$^+$ corresponding to the mass of aglycone solasodine (Fig. 3). Thus, compound **11** was identified as solasodine-hexosyl-rhamnosyl-rhamnosyl (solamargine), and the structure was further confirmed as solamargine by comparison with the retention time and mass spectrum of authentic standard. Compound **7** (RT 18.3 min) was identified in like manner and speculated to be solasonine (solasodine-hexosyl- hexosyl-rhamnosyl) as among the most reported solasodine glycosides in the *S. nigrum* complex. The elution pattern and mass spectra of these two compounds are also in agreement with the literature [23].

3.1.3. *Saponins*

Saponin identification was primarily based on mass spectrum interpretation and literature review. Compound **28** (RT 34.4 min), for example, had a molecular ion peak at 1197 *m/z* [M+H]$^+$, which occasionally featured adducted ions at 1219 *m/z* [M+Na]$^+$ and 1237 *m/z* [M + Na + H_2O]$^+$. The parent ion underwent a loss of hexosyl moiety to generate sodium adducted fragment ion 1057 *m/z* [M-hexosyl + Na]$^+$, whose remaining saccharide unites were then successively cleaved off to generate the fragment ions at 903 *m/z* [M-hexosyl-xylosyl]$^+$, 741 *m/z* [M-hexosyl-xylosyl -hexosyl + H]$^+$, 579 *m/z* [M-hexosyl-xylosyl-hexosyl-hexosyl + H]$^+$, and finally the aglycone ion at 417 *m/z* [M-hexosyl-xylosyl-hexosyl-hexosyl-hexosyl + H]$^+$, which corresponded to the mass of tigogenin as extensively found in *Solanum* complex [6,8,24]. Thus, compound **28** was identified as tigogenin conjugate with four hexosyls and one xylosyl. The other saponins were identified in similar manner. In addition, the structures of aglycones of diosgenin and tigogenin were further confirmed by comparison with authentic standards after acid-assisted hydrolysis. The representative chromatograms are shown in (Fig. 2B and C).

3.2. *Quantification of total glycoalkaloid aglycone*

As the toxicity of glycoalkaloids has long been the major concern in the consumption and promotion of *S. nigrum* complex, the glycoalkaloids were quantified as the top priority to shed light on the potential consumption risk. Given the potential existence of occasionally occurring solasodine

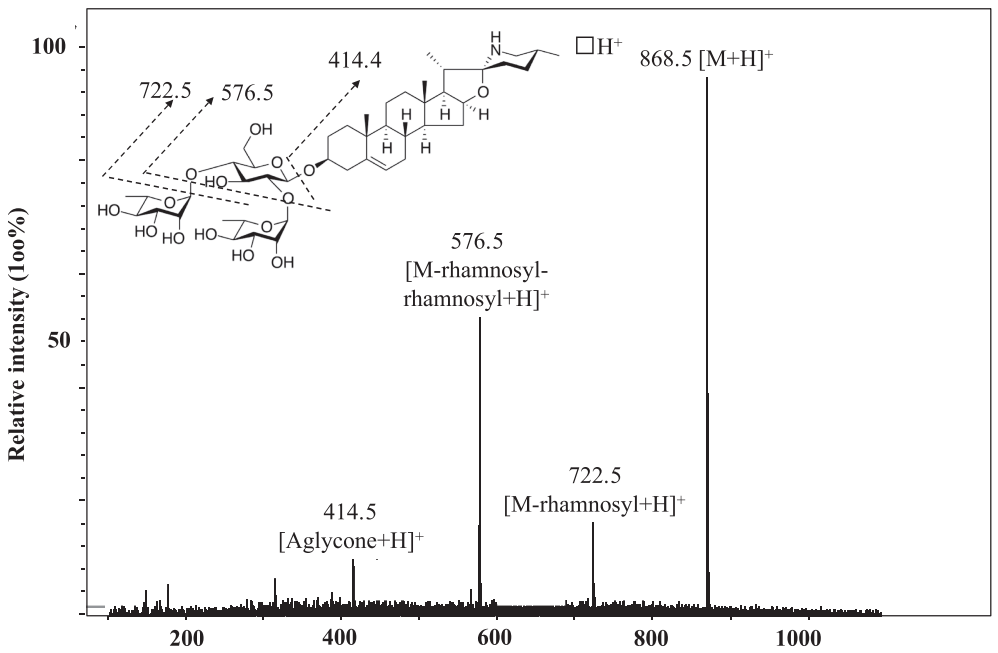

Fig. 3 – Mass spectrum of solamargine (compound 11 in Fig. 2A) with structure and MS fragment pathway.

glycosides with varied oligo/monosaccharide moieties below the detection limit, and that the aglycone part was the major source of glycoside bioactivity, in this work, the plant samples were hydrolyzed during extraction to yield the uniform aglycone solasodine as a reflection of glycoalkaloids content. Using the method developed here, the glycoalkaloid positive samples indicated in the phytochemical profiling were hydrolyzed and analyzed using MS detection under SIM mode. The calibration curve using solasodine standard was $Y = 16{,}578X+2{,}96{,}833$, $R^2 = 0.9943$, with a linearity range of 4.7 ng/mL ~47 μg/mL. The LOQ was 4.7 ng/mL, and LOD was 2.3 ng/mL. The recovery rate at ~50%, ~100% and ~200% level was 102.9 ± 25.8%, 81.5 ± 13.3%, 73.9 ± 3.0%, respectively, with each recovery level triplicated. The quantification result was summarized in Table 3.

The highest concentration of total alkaloid aglycone was found in *S. villosum* at a concentration less than 50 μg/g DW, or ~100 ug/g DW in terms of glycosylated forms, which was more than six times higher than the reported 1.62 ± 0.02 mg/100 g DW in *S. nigrum* L. var. *virginicum* of Nigeria [20]. Assuming 90% moisture content in fresh vegetables, glycoalkaloid concentration was at maximum around 1 mg/100 g fresh weight (FW). In order to evaluate the potential risk of consumption, this level was compared with *Solanum melongena* or eggplant, a globally consumed vegetable well known to contain two principle glycoalkaloids, solasonine and solamargine, a same or similar composition as that found in AIVs in this study. An extensive study revealed a safe consumption of eggplants at glycoalkaloids levels ranging from 6.25 to 20.5 mg/100 g FW [25,26], which was significantly higher than the maximum glycoalkaloid level found in the AIVs in this research ($p < 0.001$). Given the low concentration of glycoalkaloids present in the leaves of the nightshades, results suggest that the consumption of these leafy AIVs are safe.

3.3. β-carotene and vitamin E analysis

β-Carotene and vitamin E in nightshade extracts were identified and quantified against standards using UV detection at 454 nm and 293 nm, respectively. Quantification results were summarized in Table 3. β-Carotene in all four species was higher than the average level of a wide scope of household fresh vegetables reported by USDA National Nutrient Database (Figure S1 and Table S1 of the supporting information). A total of 10 entries were identified to be a source of vitamin A (Table 3) based on retinol equivalent (RE) according to standards published by Codex Alimentarius [27,28]. The averaged β-carotene content across the five accessions of *S. nigrum* was 81.1 ± 40.7 μg/g DW. Except for *S. nigrum* PI 306400, all the other accessions contained higher level of β-carotene than that reported 4.66 ± 0.02 mg/100 g DW or 46.6 ± 0.2 μg/g DW in a nutritive study of *S. nigrum* L. *var. virginicum* of Nigeria [20]. Up to 141.7 ± 11.7 μg/g DW, *S. nigrum* PI 312110 possessed the highest content in all accessions of *S. nigrum* and the other three species. In *S. scabrum*, SS 49 was found to contain the most abundant source of β-carotene with up to 96.0 ± 2.8 μg/g DW. The mean level in all eight accessions of *S. scabrum* was 79.4 ± 15.2 μg/g DW, nearly twice the level reported by Mibei at el. at 46 ± 2.5 μg/g DW [29]. The β-carotene level in the single accession of *S. americanum* was 95.8 ± 3.8 μg/g DW, less than a fifth of the reported 52.1 ± 3.6 mg/100 g DW in the literature [30]. In contrast, the β-carotene level of *S. villosum* was found to be high at 138.1 ± 4.0 μg/g DW and this is the report of β-carotene from this species.

Vitamin E content in all four species, except in a few entries, was among the top 25% when compared with the USDA National Nutrient Database (Fig. S1 and Table S1 of the supporting information). In the five accessions of *S. nigrum*, vitamin E content reached 150.3 ± 59.9 μg/g DW on average. The accession *S. nigrum* PI 312110, was found not only to be high in β-carotene, but also the richest source of vitamin E among all accessions of the four studied species at 229.7 ± 19.9 μg/g DW. In the eight entries of *S. scabrum*, the average level was 126.2 ± 44.3 μg/g DW, with BG 16 having the highest concentration of vitamin. The content in the one accession of *S. americanum* was 145.5 ± 5.2 μg/g, and in the single accession of *S. villosum* was 114.3 ± 5.0 μg/g.

3.4. Total polyphenol content and antioxidant activity

The TPP level and antioxidant activity values were summarized in Table 3. The TPP level in the five accessions of *S. nigrum* averaged 10.07 ± 2.40 mg GAE/g DW, and in the eight accessions of *S. scabrum* was 10.09 ± 1.80 mg GAE/g DW. *S. scabrum* PI 643126 and Grif 14198 was reported in another study to contain free phenolic acids at a level of 12.83 ± 0.41 mg GAE/g DW and 15.65 ± 1.08 mg GAE/g DW, respectively, which was correspondingly 65% and 42.5% higher than the TPP level found in this study [31].

Antioxidant activity in the five accessions of *S. nigrum* was averaged to be 19.6 ± 3.9 mg TE/g DW, and in the eight accessions of *S. scabrum* at 20.2 ± 2.8 mg TE/g DW. In comparison, Jimenez-Aguilar using AAPH radical reported the antioxidant activity of *S. scabrum* PI 643126 and Grif 14198 to be 34.5 ± 1.95 μmol TE/g FW and 36.17 ± 1.47 μmol TE/g FW, respectively, or 50.15 ± 2.83 mg/g DW and 51.35 ± 2.09 mg/g DW in accordance [31]. Among samples in this study, there was a close correlation between TPP content and antioxidant capability with $R^2 = 0.95$ ($n = 15$), suggesting that either the TPP content or ABTS antioxidant activity may be used for quality control purpose.

4. Conclusion

Phytochemical screening by HPLC/ESI-MS showed that all four *Solanum* species contained abundant quantities of flavonoids and saponins. All 15 sources from the four *Solanum* species showed high levels of β-carotene, vitamin E and total polyphenol level and exhibited high antioxidant activity, suggesting that edible nightshades are nutrient rich and would be of health benefit. Importantly, as sub-Saharan Africans consume the leaves and not the fruits which have been long associated with anti-nutrient Solanaceous glycoalkaloids, no glycoalkaloids were detected except in two of the studied accessions, one from *S. nigrum* and another from *S. villosum*, where total solasodine aglycone was quantified after acid hydrolysis to be less than 50 ug/g DW, or 1 mg/100 g FW in terms of glycosylated forms. The potential consumption risk at this level is low as judged by comparison with that of

globally consumed *Solanum* plant eggplant, whose glycoalkaloids were extensively studied. The glycoalkaloid content across all four species was found to be safe for consumption. The edible nightshades as part of a larger group of African indigenous vegetables were nutrient dense, possessing potential health benefits, and can be consumed without concern of glycoalkaloid related toxicity. Such results provide support for the promotion of their cultivation, marketing and consumption to provide available and accessible nutrient rich vegetables to improve household nutrition. The production of such vegetables could also provide income generating opportunities for those growers entering into the informal or formal markets.

Conflicts of interest

All contributing authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfda.2017.10.005.

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