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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, '*Solanum 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,

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