Alkaloid analysis of *Solanum nigrum* indigenous to Kenya

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Summary

The production and consumption of African indigenous vegetables (AIVs) can generate income opportunities for African rural small holder famers and can improve the health and nutrition of the family and communities by providing increased access and availabilty of fresh vegetables into their diets. Yet, AIVs can also contain natural compounds that exhibit antinutritive and/or toxicity. As we dive into the positive nutritional composition of AIVs in our HortCRSP project by examining the mineral, vitamin and phtyochemical compositions, we also wanted to develop protocols that could be used in the field and allow the rapid detection of potential toxic compounds (e.g. presence/absence of alkaloids). Here, we detail the beginnings of a method for alkaloid detection using TLC in black nightshade grown in Kenya. Results indicate that the α-solanine alkaloid was exceptionally low and not a health concern, yet all the other glycoalkaloids need to be identified.

1. Introduction

The genus *Solanum* includes important food crops such as the potato (*S. tuberosum*), tomato (*S. lycopersicum*) and eggplant (*S. melongena*), as well as black nightshade (*S. nigrum and other S. spp.*). The African nightshades are among the most popular leafy green vegetables in sub-Sahara Africa and a focus in our African Indigenous Vegetable HortCRSP project. Because many *Solanums* are known to contain glycoalkaloids, with α-solanine as the major alkaloid, we sought to develop a rapid protocol that can be used in the field to detect the presence of alkaloids and to ascertain whether the varieties being promoted and grown contain alkaloids in the leaves. To monitor the toxic alkaloid level for food safety, the alkaloids in *S. nigrum* were screened using thin layer chromatography (TLC) with visualization of the alkaloids using Dragendorff's reagent. The samples were then analyzed by HPLC in comparison with an authenticated α-solanine standard.

Fig. 1. *Solanum nigrum*

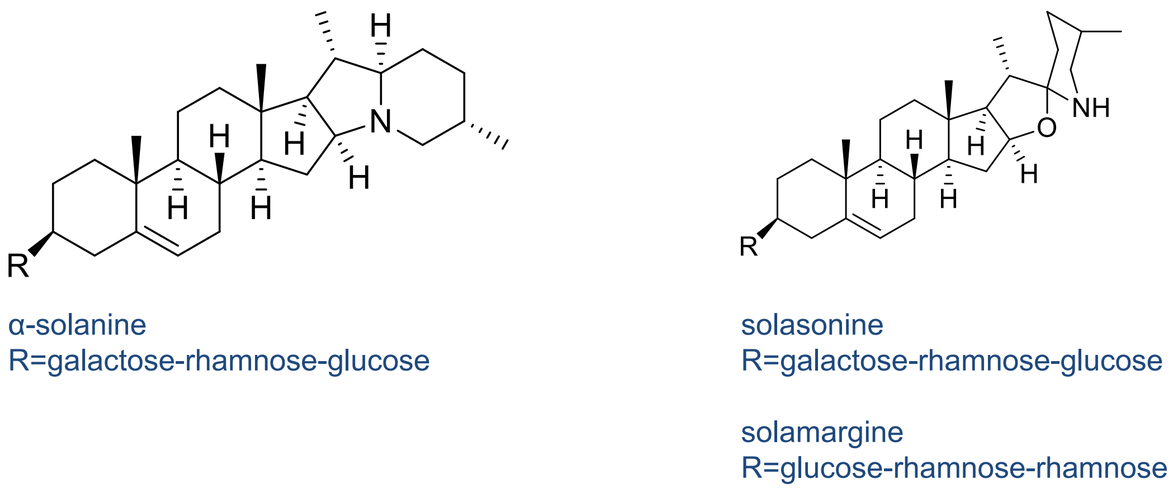

Fig. 2. Major alkaloids found in *Solanum nigrum.*

References

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2. Materials and methods

Samples.

Black Nightshade (Managu) *Solanum nigrum* leaf (AIV 12060) and Black Nightshade (Managu) *Solanum nigrum* leaf (AIV 12061) were provided by Mace Foods, Eldoret, Kenya, one of our private sector partners who specialize in dried AIVs. Our role is to assist in developing their nutritional labels for their AIV products, thus using science to support market expansion.

TLC sample preparation.

Around 500 mg of sample was extracted in 10 mL of 5% AcOH (aq., v/v) by sonication for 20 min and centrifuged. The supernatant was then passed through a preconditioned Varian C_{18} solid phase extraction cartridge (pre-conditioned with 10 mL of 40% MeOH) to elute the glycoalkaloids with 10 mL of 100% MeOH. The solvent was removed *in vacuo* and the extract re-dissolved in 500 μL MeOH.

TLC analysis.

The samples were spotted using a capillary on a silica G TLC plate, dried with a heat gun and chromatographed with 65:30:5 $CHCl_3$: MeOH: 2% NH_4OH (aq., v/v). α-solanine (1 mg/mL) standard was used as a positive control. The plates were dried with a heat gun and sprayed with Dragendorff's reagent. Heated the TLC plate using heat gun and the alkaloids were visualized as orange spots.

HPLC sample preparation.

Around 500 mg of sample was extracted in 5 mL of 5% AcOH (aq., v/v) by sonication for 20 minutes and centrifuged for HPLC analysis.

HPLC analysis.

Agilent 1100 Series LC/MS equipped with autosampler, quaternary pump system, DAD detector, degasser and HP ChemStation software. The column used was a Varian Polaris 5 C18-A, 4.6 x 250 mm.

HPLC conditions.

The mobile phase consisted of solvent A (20 mM KH_2PO_4) and solvent B (ACN) for the following gradient at a flow rate of 1.0 mL/min: 20 to 25% B 0-10 min, 25 to 30% B 10-15 min, 30% B 15-20 min, 30 to 100% B 20-25 min, 100% B 25-35 min, 100 to 20% B 35-40 min with a 10 min equilibration. UV absorbance was measured at 205 nm. A calibration curve was constructed for α-solanine and glycoalkaloid content calculated with a correction factor of MW ratio.

3. Results and Discussion

The *Solanum nigrum* samples examined in this study were found to have alkaloid levels that are in the lower range of what is reported in the literature (1, 2). These samples were found to have an unusually low amount of α-solanine, which was previously reported as major alkaloid in nightshade. Guidelines for potatoes recommend limiting glycoalkaloid content to 200 mg/kg fresh weight, which is well above the levels found in *S. nigrum* species analyzed (3).

Fig. 3. TLC separation showing the α-solanine standard (left) and a representative *Solanum nigrum* extract (right).

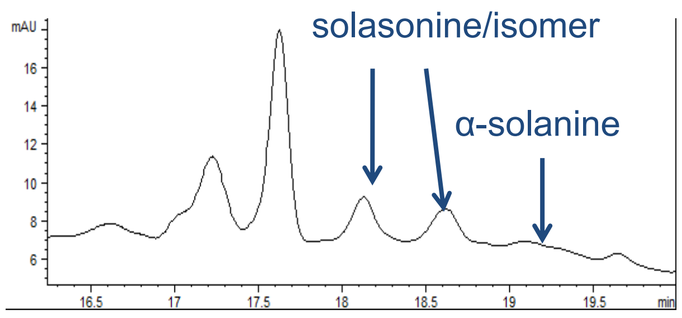

Fig. 4. UV (205 nm) chromatogram of *Solanum nigrum* (AIV 12060).

Sample	solasonine/isomer (mg/g, n=3)	solasonine/isomer (mg/g, n=3)	α-solanine (mg/g, n=3)
AIV 12060	0.103 ± 0.002	0.076 ± 0.002	trace
AIV 12061	0.098 ±0.001	0.070 ± 0.002	trace