Application of TLC and UHPLC–QTOF–MS for the identification of aqueous two-phase extracted UV–fluorescent metabolites from *Solanum retroflexum*

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1 Introduction

Solanum species are renowned for their rich diversity of metabolites. Some of the many metabolites include flavonoids and glycoalkaloids which have been reported to exhibit antioxidant and protective roles, respectively [1]. Daji *et al.* [2] investigated the phytochemical profile of *Solanum retroflexum* leaves using methanolic extracts and found a range of cinnamic acids, polyphenols and alkaloids. Though methanolic extraction has been applied quite extensively [2–6], this approach is accompanied by setbacks such as the use of toxic organic solvent which often requires large volumes for extraction solvents, making it a costly exercise. Hence, there is a dire need for effective, eco-friendly, single-step extraction techniques.

Aqueous two-phase extraction (ATPE) is one such method that offers efficient, eco-friendly quick metabolite extraction. ATPE uses salts that allow for partitioning of a green extraction solvent, usually ethanol, from water, resulting in the extraction solvent being simultaneously enriched in the desired metabolites [7]. ATPE extracted phytocompounds were reported by Mokgehle *et al.* [8] with

a range of cinnamic acids, polyphenols and alkaloids being obtained from *S. retroflexum*. Some of the ATPE extracted alkaloids include solanagin, solamargine, solasonine and β -solanine. The same authors also reported ATPE as being essential for the simultaneous extraction of multiple metabolites. Some studies have been directed at the use of thin-layer chromatography (TLC) methods for the isolation of potentially bioactive compounds from *Solanum* species. For instance, Shamsaldin *et al.* [9] applied TLC for the isolation of flavonoids from *Solanum villosum* Mill., Patel *et al.* [10] reported on the separation of alkaloids from *Solanum trilobatum* using TLC. In another study, Jadesha *et al.* [11] examined TLC for the isolation of phenolic compounds from *Solanum torvum*. Therefore, this work attempts to answer the question why a locally grown vegetable, *S. retroflexum*, in the Vhembe district (South Africa) has been a sought-after commodity for the health and well-being of communities in the area. The current work is the first attempt to separate and identify ATPE extracted ultraviolet (UV)–fluorescent metabolites present in *S. retroflexum* based on two independent chromatographic techniques, *i.e.*, as TLC and ultra-high performance liquid chromatography–quadrupole time-of-flight hyphenated to mass spectrometry (UHPLC–QTOF–MS).

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2 Experimental

The leaves of *S. retroflexum* were air-dried and ground into a fine powder with a rotating blade blender and stored in covered glass containers. The powdered leaves (2.00 g) were placed in 50 mL polypropylene tubes. To each tube, a saturated salt solution of 25 mL was added. The saturated salt concentrations used were 30% (w/V) of BaCl₂,

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Na_2HPO_4 , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, AgNO_3 , KBr and KNO_3 . Thereafter, 25 mL of absolute ethanol (99.9%) extract and 25 mL of each salt solution containing the powdered leaves were mixed, resulting in ATPE. The spontaneous formation of ATPE under the conditions stated above was also reported by *Mokgehele et al.* [8]. Portions

of $3 \times 20 \mu\text{L}$ absolute ethanolic (99.9%) top-layer extract-ant solution were spotted on the TLC plate and developed using a mobile phase of chloroform–ethyl acetate–methanol (45:40:15, V/V). The separated phytocompounds were observed on the TLC plate using a UV lamp (365 nm), scraped (Fig. 1a, b), dissolved in ethanol and analyzed on a

Fig. 1 **a** Fluorescent spots on TLC plate when chloroform–ethyl acetate–methanol (45:40:15, V/V) was as a developing solvent at 365 nm. **b** Scraped fluorescent spots from B1 to B6

UHPLC–QTOF–MS. The metabolites were separated on an Acquity HSS T3 C18 column (150 mm × 2.1 mm with particle size of 1.7 µm; Waters Corporation, Milford, MA, USA) at an oven temperature of 40 °C. The UPLC was connected to a Synapt G1 QTOF–MS detector (Waters). An injection volume of 3 µL was used with solvent A: 0.1% formic acid in Milli-Q water and solvent B: acetonitrile with 0.1% formic acid. Chromatographic separation was achieved using a 10 min gradient elution: 2% B over 0.0–1.0 min, 2%–95% B over 1.0–6.0 min, from 6.0 to 7.0 min the conditions were maintained at 95% B, the column was washed with 95–2% B over 7.0–8.0 min, re-equilibration with 2% B over a 2 min isocratic wash. The MS was configured to scan the range of 100–1000 Da with a scan time of 0.2 s. The MS data were acquired using positive electrospray ionization (ESI) mode. Chemical identification was done using KNapSack Core System online metabolite database (Version 1.200.03) [12].

3 Results and discussion

Generally, an average of six bands (B1–B6) potentially corresponding to six compounds or more was observed on the TLC plate (Fig. 1a, b) when salts such as $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, BaCl_2 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Na_2HPO_4 , KNO_3 and KBr were used during ATPE to aid the extraction of metabolites. Additionally, while the majority of the red fluorescent compounds were observed further up the TLC plate, blue fluorescent compounds were also observed at the top of the plate. *Nazir et al.* [13] also reported on fluorescent compounds in *S. lycopersicum* separated via TLC.

The UV–fluorescent TLC extracted bands (B1–B6), after dissolution in ethanol, were run on the UPLC–QTOF–MS. A chromatographic base peak at m/z 435 and retention time (t_R) of 5.4 min were observed for B2 (Fig. 2 and Table 1) and another at m/z 457 (Fig. 2 and Table 1) with a retention time (t_R) of 7.31 min for B1. The base peak at m/z 457 was identified as oleanolic acid (OA) or its isomers such as ursolic acid (UA) or betulinic acid (BA) (Table 1) (pentacyclic triterpenoid) and was also reported to have been extracted from *Scutellaria barbata* D. [14]. The chromatographic base peak at m/z 435 detected for B2 had fragments at m/z 149, 277 and 303 and was identified as quercetin 3-*O*- β -D-xylofuranoside (Fig. 2 and Table 1). Quercetin-3-*O*- β -D-xylofuranoside was also extracted from potato (*Solanum* spp.) [15] and *Vernonia* Schreb. [16]. A chromatographic appearance at retention time of 3.27 min with a base peak at m/z 415 was observed for B3 and B4 (Fig. 2 and Table 1). The fragments of the base peak at m/z 415 were at m/z 142 and 224, and hence identified as solanocapsine (Fig. 2 [B3] and Table 1). Solanocapsine was also extracted from *S. pseudocapsicum* (Jerusalem cherry) as reported by *Garcia et al.* [17].

Two chromatographic base peaks at m/z 413 were observed at B5 and indicated the presence of isomeric compounds (Fig. 2 and Table 1). The isomers had nominal masses of 413.2645 and 413.3223 and eluted at 7.46 and 7.83 min, each with fragments at m/z 133, 149, 301, 326 and at m/z 133, 327, 301, 369, and were identified as stigmaterol I and stigmaterol II, respectively (Fig. 3a and Table 1). The types (I or II) indicated isomers of stigmaterol. *Kaminski* [18] also reported on the presence of stigmaterol in *S. tuberosum*. The chromatographic appearance at 3.83 min with a base peak at m/z 1034 was observed in B4, furthermore the fragment of m/z 1034, m/z 578 was also observed as a chromatographic peak at the same retention time in B2 (Fig. 2 and Table 1).

The chromatographic base peak at m/z 1034 had fragments at m/z 263, 416, 528, 578 (Table 1). Similarly, the chromatographic base peak at m/z 578 (B2) also produced the same fragments as those of m/z 1034 (B4), suggesting that both have the same structural moiety. In addition, the chromatograms in Fig. 2 show a richness in peaks which indicated that the richness peaks from the various zones on the TLC plate could be due to a common structural backbone present in multiple compounds with similar polarities. Additionally, the richness in peaks may be a result of the presence of isomeric metabolites. From the KNapSack metabolite database, the fragment at m/z 578 and the base peak ion at m/z 1034 were identified as tomatidine galactoside ($\text{C}_{33}\text{H}_{56}\text{NO}_7$) and alpha-tomatine ($\text{C}_{50}\text{H}_{83}\text{NO}_{21}$) (Fig. 3b and Table 1), respectively [21]. Alpha-tomatidine was composed of a tomatidine aglycone unit glycosylated by four monosaccharides which included D-galactose, 2 × D-glucose and D-xylose, whereas tomatidine galactoside contained D-galactose as a saccharide [22]. Therefore, this indicated that the transition from m/z 1034 to m/z 578, which is alpha-tomatine to tomatidine galactoside, occurred with the loss of 3 monosaccharides which consisted of 2 × D-glucose and D-xylose. A chromatographic appearance at 7.42 min with a base peak at m/z 560 that produced daughter ions at m/z 376 and 443 was observed as a blue fluorescent band at B6. From Fig. 1a, the red fluorescent metabolites were dominant in zones B1–B5 while the blue fluorescent compounds were present at B6. Through KNapSack, the base peak at m/z 560 was identified as gamma-solanine. The diverse fluorescing behavior of the metabolites, for instance the blue and red fluorescent gamma-solanine and solanocapsine, respectively, on the TLC in Fig. 1a, could be due to the possibility of various structural moieties within the metabolites. Alpha-tomatine and gamma-solanine have been reported in *S. lycopersicum* (tomato) to be toxic for consumption particularly during the greening stage [21].

Fig. 2 Base peak single-ion UHPLC-QTOF-MS chromatograms of metabolites extracted using ethanol under positive ionization *via* ATPE from the leaves of *Solanum retroflexum*

Table 1 Major compounds identified by UHPLC in *Solanum retroflexum* leaf aqueous methanol extracts

Band	Compound	Chemical formula	[M + H] ⁺	Diagnostic ions	t _R (min)	λ _{max} (nm)	Plant species previously found in	References
B1	UO/OA/BO	C ₃₀ H ₄₈ O ₃	457	374, 512	7.31	210	<i>Scutellaria barbata</i>	[14]
B2	Quercetin-X (reynoutrin)	C ₂₀ H ₁₈ O ₁₁	435	149, 277, 303	5.40	352	<i>Solanum</i> spp.	[15]
B3	Solanocapsine	C ₂₇ H ₄₆ N ₂ O	415	142, 224	3.27	293	<i>S. capsicastrum</i> , <i>S. pseudocapsicum</i>	[16]
B4	Alpha-tomatine	C ₅₀ H ₈₃ NO ₂₁	1034	263, 416, 528, 578	3.83	208	<i>S. lycopersicum</i>	[19]
B5	Stigmasterol I	C ₂₉ H ₄₈ O	413	133, 301, 326	7.46	257	<i>S. chacoense</i> , <i>S. tuberosum</i>	[20]
	Stigmasterol II	C ₂₉ H ₄₈ O	413	133, 301, 369	7.83	257	<i>S. chacoense</i> , <i>S. tuberosum</i>	[20]
B6	Gamma-solanine	C ₃₃ H ₅₃ NO ₆	560	376, 443	7.42	325	<i>S. chacoense</i> , <i>S. tuberosum</i>	[21]

X = 3-*O*-β-D-xylofuranoside, UO = ursolic acid, OA = oleanolic acid, BO = betulinic acid

Fig. 3 Some of the structures detected from ATPE extracts of *Solanum retroflexum*
a stigmasterol **b** alpha-tomatine

4 Conclusion

The combined application of TLC and UPLC–QTOF–MS was shown to be useful in the identification of nine UV–fluorescent compounds from *S. retroflexum* for the first time,

with the aid of the KnapSACk metabolite database. Three UV–fluorescent alkaloids, solanocapsine (red fluorescent), alpha-tomatine (red fluorescent) and gamma-solanine (blue fluorescent) were simultaneously extracted *via* ATPE and subsequently isolated by chromatography-based methods.

The diverse fluorescing behavior of the metabolites, under UV light, was possibly due to the variation in the structural moieties of the metabolites. To date, alpha-tomatidine and gamma-solanine have generally been limited to *S. lycopersicum*. However, this study has demonstrated that both glycoalkaloids can also be found in *S. retroflexum*. The use of an environmentally friendly method, ATPE, in conjunction with TLC and UPLC–QTOF–MS has shown to be an efficient method for the simultaneous extraction of UV–fluorescent metabolites from *S. retroflexum* and may prove vital for the well-being of man. Though this study was an untargeted approach with a tentative identification of UV–fluorescent metabolites from *S. retroflexum*, future studies may be directed at the isolation and comprehensive identification of isomers (ursolic acid, betulinic acid and oleanolic acid) by incorporation of derivatization agents.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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