

Research Article

Phytochemical Composition of *Solanum retroflexum* Analysed with the Aid of Ultra-Performance Liquid Chromatography Hyphenated to Quadrupole-Time-of-Flight Mass Spectrometry (UPLC-qTOF-MS)

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Solanum retroflexum (nightshade) is an edible plant that is consumed in some regions of South Africa. Its leaves are a good source of vitamins, proteins, and minerals. It appears that there is no scientific report about the phytochemical composition of *S. retroflexum*. Here, ultra-performance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UPLC-qTOF-MS) technique was used to achieve an untargeted metabolite fingerprinting of this plant. A total of 30 phytochemicals, including alkaloids, flavonoids, and cinnamic acids derivatives, were identified from the methanolic leaf extracts. The concentration of solvent did not affect the type of compounds extracted. The extraction resulted in qualitative yields of molecules such as quercetin-3-rutinoside, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, 3-caffeoylquinic acid, 5-caffeoylquinic acid, and 3, 4-di-caffeoylquinic acid. The present study confirms the presence of phytochemical compounds in *S. retroflexum* similar to other *Solanum* plants.

1. Introduction

Solanum plants (family Solanaceae) have been widely explored for their phytochemical constituents. These bioactive compounds have been isolated from various parts such as the leaves, fruits, and roots [1–3]. The presence of various phytochemicals in *Solanum* plants has allowed for their use in medicine and food as functional foods or dietary supplements [4]. The type of phytochemical compounds found in *Solanum* plants vary with species, plant part, and the extraction method [5]. The presence of tannins, flavonoids, alkaloids, glycosides, steroids, and terpenoids has been reported in *S. nigrum* L. leaves, fruits, and stem [3]. Phenolic compounds such as chlorogenic acid, caffeic acid, rosmarinic acid, gallic acid, and flavonoids have been identified together with alkaloids in *S. corymbiflorum* leaves [2]. The

presence of some compounds such as alkaloids in food can cause toxicity to humans depending on dosage. At low doses, the intake of glycoalkaloids may cause gastrointestinal disturbance such as vomiting, diarrhea, and abdominal pain, while at high doses, it may lead to serious complications such as rapid pulse and in some cases, coma and death [6].

Extraction of phytochemicals from plants is generally done with conventional extraction methods such as the use of organic solvents. However, there are concerns about the potential health threatening effects of organic solvents. Despite this, the use of organic solvents such as methanol is known to produce good extraction yields. In addition, aqueous methanol has been reported to extract higher amounts of phenolic compounds compared to absolute methanol. Quadrupole time-of-flight mass spectrometry (qTOF-MS) fingerprinting method is one of the improved

methods that has been proved to generate stable and reproducible results [7]. Coupling the HPLC with MS does not only offer excellent sensitivity and selectivity but also allows for determination of unknown and known phytochemical compounds [8]. The qTOF-MS fingerprinting method has been proved to generate stable and reproducible results, particularly in the identification of chlorogenic acid [9]. It appears that there is no scientific report on the phytochemistry of *Solanum retroflexum* despite the fact that the people in Venda region, Limpopo province, South Africa, consume the leaves as food. Therefore, the aim of this research was to investigate the phytochemical composition of *S. retroflexum* leaf extracts with the aid of UPLC-qTOF-MS.

2. Materials and Methods

2.1. Plant Collection. *Solanum retroflexum* leaves were collected from the Venda region, Limpopo province, South Africa, between September and October 2015. The leaves were air-dried under shade and stored in airtight containers at room temperature for further use.

2.2. Extraction of Metabolites. Three different types of sample extracts were obtained by using aqueous methanol (40%, 60%, and 80%, methanol/water, v/v) solution as solvent. *Solanum retroflexum* leaf samples were extracted following the method described by Abu-Reidah et al. [10] and Ramabulana et al. [11], with slight changes. The grounded (2.0 g) leaves of *S. retroflexum* were extracted with different concentrations of aqueous methanol and sonicated at room temperature for 15 minutes. The resultant mixture was centrifuged for 10 minutes at 5000 g and evaporated using a Buchi rotary evaporator to approximately 1 mL. The extracts were then dried to completeness overnight, dissolved again in methanol, and filtered through 0.22 μm syringe filters and kept at -20°C prior to analysis.

2.3. Ultrahigh-Performance Liquid Chromatography and Mass Spectrometry. Chromatographic separation was conducted according to the procedure described by Ramabulana et al. [11] using an Acquity UHPLC connected to a Synapt G1 qTOF-MS detector (Waters Corporation, MA, USA). The mobile phase consisted of formic acid (0.1%) in deionised water (solvent A) and acetonitrile with 0.1% formic acid (Romil Pure Chemistry, Cambridge, UK) (solvent B). Chromatographic separation was achieved using a 30 min gradient elution method consisting of the following settings: the initial conditions were 98% A at a flow rate of 0.4 mL/min. The initial conditions were kept constant for 1 min. Conditions were changed to 97% A at 3 min, reduced slightly to 92% A at 4 min, held for 21 min, and then changed to 50% A at 25 min. Conditions were changed to 5% A at 26 min and kept for 2 min and finally returned to initial conditions of 98% A at 28 min and kept constant for another 2 min to allow reequilibration before the next run. Elution was monitored using a photodiode-array detector (PDA) collecting 20 spectra per second between the 200 and 500 nm range. For mass spectrometry, the acquisition parameters

discussed by Ramabulana et al. [11] were followed. Briefly, MS data were acquired using both positive and negative ESI modes. The MS was configured to scan the range of 100–1000 Da with a scan time of 0.2 s. After series of optimization, the following settings were found to be optimal: capillary voltage of 2.5 kV, sample cone potential of 30 V, source temperature of 120°C , desolvation temperature of 450°C , cone gas flow of 50 L/h and desolvation gas flow of 550 L/h, and multichannel plate detector potential of 1600 V. In order to achieve efficient fragmentation to aid during identification, data were collected using collision energy ramp of 10–30 eV and, when necessary, a higher collision energy ramp of 165–60 eV was also used. Unless stated, otherwise only ESI negative data are reported herein.

3. Results and Discussion

3.1. Methanol Extraction of Phytochemicals. Different concentrations of methanol (40%, 60%, and 80%, methanol/water, v/v) were used in this study to understand different degrees of the extractive potency of different concentrations of the solvent. Screening of metabolites was achieved by an untargeted UPLC-qTOF-MS at positive and negative ionization modes. For efficient metabolite identification, MS fragmentation patterns were used in conjugation with standards. The methanol extracts of *Solanum retroflexum* generated using the different concentrations of aqueous methanol resulted in very similar phytochemical composition (Figure 1). A total of 30 metabolites were identified, and these include chlorogenic acids (CGAs), flavonoids, and alkaloids as shown in Table 1.

3.2. Chlorogenic Acids. Chlorogenic acids are phenolic acids that result from the esterification of quinic acid (QA) and cinnamic acid (CA) derivatives including caffeic, sinapic, and ferulic acid [12]. In this study, chlorogenic acids were identified chromatographically with the aid of our recently developed in-source collision induced dissociation method. Generally, CGAs result in very similar fragments characterised by the presence of Q1 [quinic acid-H], C1 [caffeic acid-H], Q2 [quinic acid- H_2O] and C2 [caffeic acid- CO_2] fragment ions [13]. Four peaks at m/z 353 with region isomers and geometric isomers of caffeoylquinic acid (CQAs) were detected in the extracts of *S. retroflexum* leaves (Figure 2). The CQA derivatives, the most common form of CGA, are formed from the esterification of quinic acid to one or more caffeic acid unit. The metabolites from CGAs and their isomers (positional) can be differentiated mainly by fragmentation patterns into 3 CQA, 4 CQA, and 5 CQA [14].

Chromatographically, it is easy to identify CGA and also differentiate between the *trans* and *cis* isomers because of the order in which they elute [9]. It is worth noting that these isomers (*trans* and *cis*) are known to have similar fragmentation patterns [15]. Here, both the *trans* and *cis* isomers were identified (Table 1). This can be attributed to too much exposure of the leaves to UV rays since the *S. retroflexum* came from the northern part of South Africa that is known to be very hot. Ultraviolet rays are known to convert *trans* to *cis* isomer [16]. The presence of these *cis* isomers is important

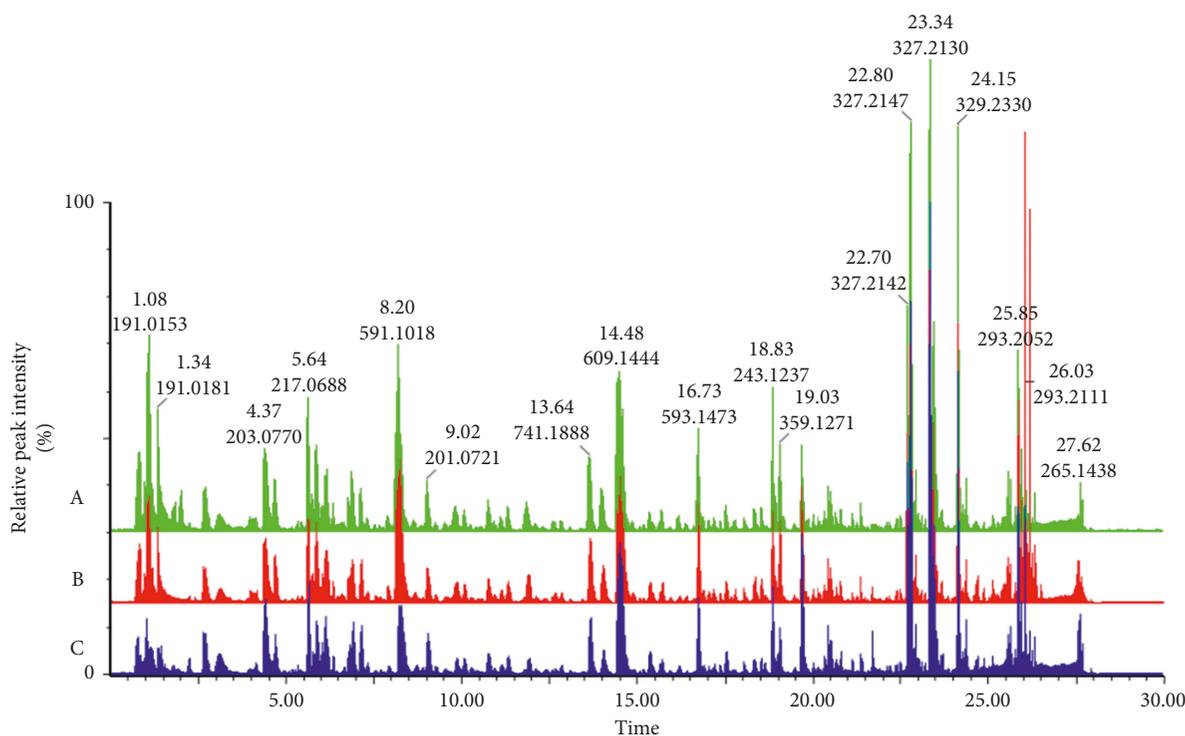


FIGURE 1: UHPLC-qTOF-MS chromatogram of 40% (a), 60% (b), and 80% (c) aqueous methanol *Solanum retroflexum* leaf extracts showing peaks detected in the negative- and positive-ionization mode.

since they are known to possess biological activities such as antioxidant activity, antimicrobial activity, antiviral activity, and antidiabetic activity [17].

The presence of CGAs in *Solanum* leaves has previously been reported in the Solanaceae family but not in *S. retroflexum*. For instance, CGAs were identified in *Lycium intricatum* methanolic leaves extract [10]. *Solanum corymbiflorum* leaves extracted with ethanol were also found to contain high amounts of CGAs [2]. Previous research has shown that CGAs may exhibit antibacterial, antiviral, and antioxidant activities [18]. In addition, anti-inflammatory activities of CGAs possibly by modulating a number of important metabolic pathways have been reported elsewhere [19]. Chlorogenic acids are also found in beverages such as tea, coffee, and wine. They could be responsible, in part, for the health promoting benefits of such food products.

Apart from the CQAs, other monoacyl CGA that are not ferulic but coumaric and ferulic were identified in this study. Also, diacyl CGAs which are very important molecules with good biological activity, such as being natural antioxidants in food or nonfood products, were observed.

3.3. Characterisation of Caffeoylquinic Acids. Molecules 1, 2, and 3 were identified as 3CQA, 4CQA, and 5CQA, respectively (Table 1). Molecule 1 at retention time 9.8 min produced a precursor ion at m/z 353 identified as *cis*-3CQA. This molecule produced fragments ions at m/z 179, 191, and 135 characteristic of quinic acylated at 3 positions with caffeoylquinic acids, thus 3CQA [20]. Molecules 2 and 3

were identified as *trans*-4CQA and 5CQA with a precursor ion at 191, 179, 173, and 135. Molecules 4 and 5 were identified as *cis*-4CQA and 5CQA. The CQAs are the most researched amongst the CGAs because they widely occur in nature [9]. It was also noted that *cis*-5-CQA showed a high intensity than *cis*-3-CQA (Figure 2). It has also been reported that the same molecule is induced by activators responsible for plant defence response [21].

3.4. Characterisation of Feruloylglycoside. Feruloylglycosides have a molecular weight (M_r) of 356, and they produce a precursor ion at m/z 355. Here, only one molecule was identified, and it had a base peak at m/z 175 and 160 ([ferulic acid-H-H₂O]) (Table 1). The fact that feruloylglycoside molecule is biosynthesised in *Solanum retroflexum* leaves suggests a remarkable biochemical feature of this plant.

3.5. Characterisation of Di-caffeoylquinic Acid and Caffeoylquinic Acid-glucoside. Both *di*-caffeoylquinic acid and caffeoylquinic acid glucoside were identified with a precursor ion at m/z 515 (Table 1). These molecules can be identified separately using their accurate masses and fragmentation patterns [22]. Here, molecules 7–13 were identified as either *di*-CQA or CQA-glycoside. Previous research indicate that *di*-CQA and CQA glycosides have an average m/z of 515.1463 (C₂₅H₂₃O₁₂) and 515.1292 (C₂₂H₂₇O₁₄), respectively, based on accurate mass [22]. Molecules 7 and 8 were identified as *trans*- and *cis*-CQA-glycoside. The CQA glycoside produced unique ions that did not occur in the

TABLE 1: Compounds identified with UHPLC in *Solanum retroflexum* leaf aqueous methanol extracts.

| Mol. no. | Retention time (min) | Mass | Compound name | Diagnostic <i>m/z</i> ions |
|----------|----------------------|------|------------------------------------|----------------------------|
| 1 | 9.8 | 353 | 3-CQA | 191, 179, 135 |
| 2 | 5.6 | 353 | <i>trans</i> -4-CQA | 191, 135, 173, 179 |
| 3 | 5.1 | 353 | <i>trans</i> -5-CQA | 191, 135 |
| 4 | 6.0 | 353 | <i>cis</i> -4-CQA | 191, 135, 173, 179 |
| 5 | 5.9 | 353 | <i>cis</i> -5-CQA | 191, 135 |
| 6 | 6.5 | 355 | Feruloylglycoside | 160,175 |
| 7 | 5.3 | 515 | <i>trans</i> -5-CQA glycoside | 191, 135 |
| 8 | 6.4 | 515 | <i>cis</i> -5-CQA glycoside | 191, 135 |
| 9 | 8.9 | 515 | 3, 4- <i>di</i> -CQA | 191, 179, 173, 135 |
| 10 | 9.1 | 515 | <i>cis</i> -3, 5- <i>di</i> -CQA | 191, 179, 135 |
| 11 | 9.3 | 515 | <i>trans</i> -3, 5- <i>di</i> -CQA | 191, 179, 135 |
| 12 | 9.5 | 515 | <i>trans</i> -4, 5- <i>di</i> -CQA | 191, 179, 135 |
| 13 | 10.5 | 515 | <i>cis</i> -4, 5- <i>di</i> -CQA | 191, 179, 135 |
| 14 | 5.3 | 337 | 3- <i>p</i> -CoQA | 177 |
| 15 | 6.8 | 337 | <i>trans</i> -5- <i>p</i> -CoQA | 191 |
| 16 | 7.5 | 337 | <i>cis</i> -5- <i>p</i> -CoQA | 191 |
| 17 | 8.7 | 339 | Coumaroyl-hexose | 223, 164, 149 |
| 18 | 7.4 | 337 | 4- <i>p</i> -CoQA | 173 |
| 19 | 7.4 | 367 | <i>trans</i> -5-FQA | 191 |
| 20 | 8.0 | 367 | <i>cis</i> -5-FQA | 191 |
| 21 | 10.7 | 367 | 4-FQA | 173 |
| 22 | 10.3 | 367 | 3-FQA | 193, 134 |
| 23 | 8.1 | 609 | Quercetin-3-rutinoside | 300 |
| 24 | 9.1 | 447 | Kaempferol-3-glucoside | 284 |
| 25 | 8.4 | 463 | Quercetin-7-glucoside | 300 |
| 26 | 8.8 | 593 | Kaempferol-3-0-rutinoside | 285 |
| 27 | 9.0 | 623 | Isorhamnetin-3-0-rutinoside | 315 |
| 28 | 8.7 | 447 | Quercetin-3-rhamnoside | 300 |
| 29 | 20.3 | 884 | Solasonine | 722, 576, 414 |
| 30 | 20.1 | 868 | Solamargine | 722, 576, 414 |

Note. CQA, caffeoylquinic acid; *di*-CQA, *di*-caffeoylquinic acid; *p*-CoQA, *p*-coumaroylquinic acid; FQA, feruloylquinic acids; mol no., molecule number; min, minutes; *m/z*, mass-to-charge ratio; DNP, dictionary of natural products 2015 [37]; KMI, KNAPSAcK Metabolites Information 2015 [38].

di-CQA MS spectra. These were at *m/z* 341 ([caffeoyl glucoside-H]⁻) and/or at *m/z* 323 (caffeoyl glucoside-H₂O)⁻). Recently, the hierarchical fragmentation schemes of similar molecules have been reported [23]. Their work noted the formation of a glycoside by CQA through an ether bond at either C-3 or C-4 on the aromatic caffeoyl ring. Molecules **9–13** were identified as *di*-CQAs (3, 4 *di*-CQA (**9**), *trans*- and *cis*-3, 5 *di*-CQA (**10** and **11**), and *trans*- and *cis*-4, 5 *di*-CQA (**12** and **13**).

3.6. Characterisation of *p*-Coumaroylquinic Acid and Coumaroyl-hexose. The *S. retroflexum* leaf extracts showed precursor ions at *m/z* 337, and these were identified as *p*-coumaroylquinic acid (Mr of 338) (Table 1). These ion peaks were identified as 3-*p*CoQA (**14**), *trans* 5-*p*C_OQA (**15**), *cis*-5-*p*C_OQA (**16**), and 4-*p*-C_OQA (**18**). The presence of *p*-CoQA is not surprising since it has also been identified in leguminous plants and birch trees [24]. Molecule **17** was identified as Coumaroyl-hexose with a precursor ion at *m/z* 339 and product ions at 223, 164 and 149.

3.7. Characterisation of Feruloylquinic Acids. The identification of feruloylquinic acids is based on that fact that they have a Mr of 368. In this study, molecules harbouring ferulic

acid moieties were observed. This was similar to what occurred with *p*CoQA and CQA. Here, the peaks identified as feruloylquinic acids were *trans*-5-FQA (**19**), *cis*-5-FQA (**20**), 4-FQA (**21**), and 3-FQA (**22**) (Table 1).

3.8. Characterisation of Flavonoid Derivatives. A total of six flavonoids **23, 24, 25, 26, 27,** and **28** were detected and characterised in *S. retroflexum* by QTOF-MS method presented herein (Table 1). Molecule **23** at retention time 8.12 min produced a precursor ion at *m/z* 609, and its fragmentation pattern was found consistent with that of quercetin-rutinoside in the literature [25]. Briefly, the product ions of molecule **23** were primarily caused by the exclusion of a rutinoside sugar moiety, which resulted in an intense peak at *m/z* 300. Importantly, quercetin is reported to be a crucial dietary flavonoid that possesses health benefits such as suppressing chronic diseases [26]. This has made it useful for the treatment of certain chronic diseases. Furthermore, quercetin has other biological potential associated with protection of liver cells and prevention of suppressing oxidation of the haemoglobin as a result of its antioxidant activity [27].

Molecule **24** had a precursor ion at *m/z* 447 [M-H]⁻, and it was identified as kaempferol-3-glucoside (Table 1). It produced fragment ions at *m/z* 284 which is thought to be

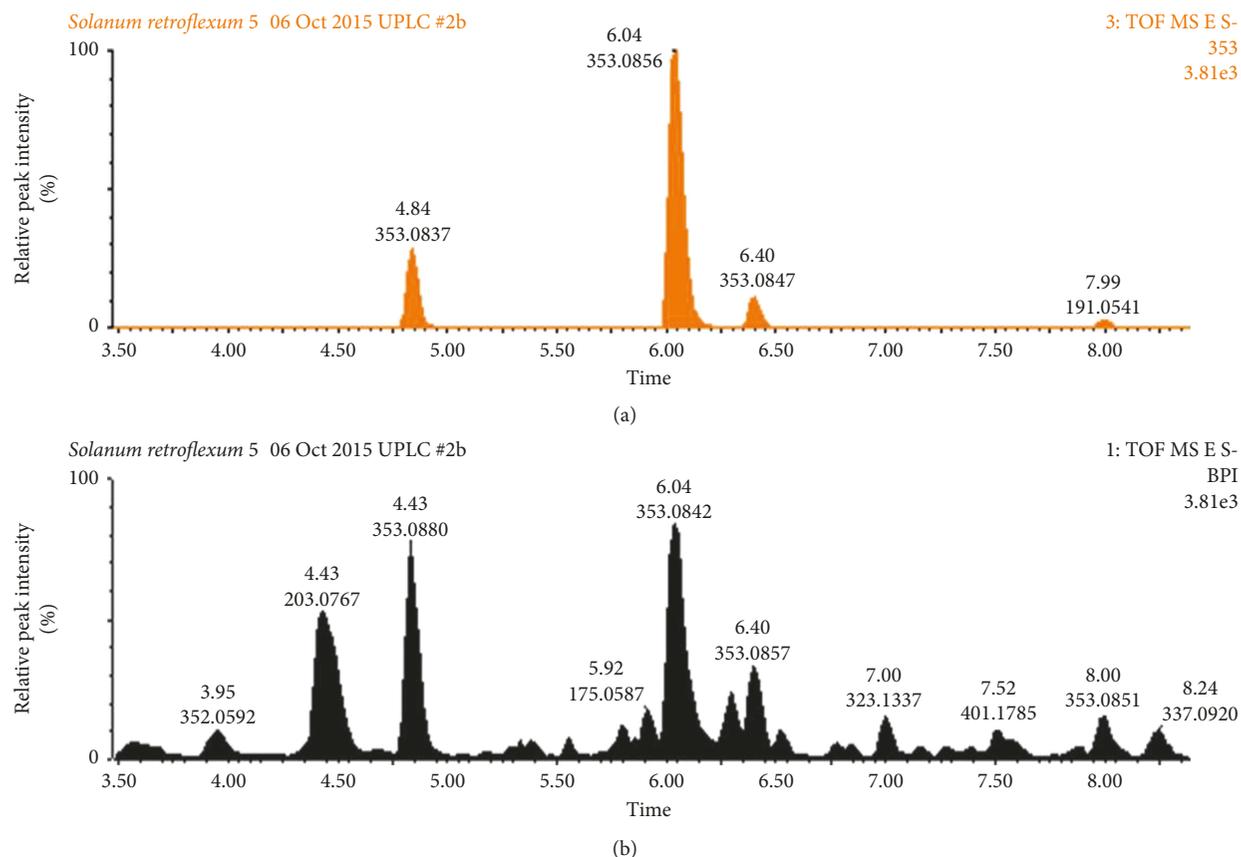


FIGURE 2: UHPLC-qTOF-MS chromatogram showing single-ion chromatogram (a) and the base peak in chromatogram (b) of chlorogenic acid detected in the negative-ionization mode.

due to the loss of a hexose moiety [28]. Molecule **25** was identified as quercetin-7-glucoside because it had a precursor ion at m/z 463 $[M-H]^-$ and a fragment ion produced at m/z 300. Molecule **26** was identified as kaempferol-3-0-rutinoside with a precursor ion at m/z 593 $[M-H]^-$ and a fragment ion at m/z 285, indicating the elimination of a rutinoside moiety. Such a fragment pattern has been reported elsewhere [29]. Molecule **27** had a precursor and fragment ions at m/z 623 $[M-H]^-$ and m/z 315, respectively. It was identified as isorhamnetin-3-0-rutinoside. According to Makita et al. 2016 [25], the absence of a rutinoside moiety makes it to represent an isorhamnetin aglycone. Molecule **28** was identified as quercetin-3-rhamnoside because of the presence of a precursor ion at m/z 447 $[M-H]^-$ and the fact that it produced a fragment ion at m/z 300.

In foods, flavonoids occur as O-glycosides with sugar bound at C₃ position. The structures of quercetin-3-rutinoside, kaempferol-3-glucoside, quercetin-7-glucoside, kaempferol-3-0-rutinoside, isorhamnetin-3-0-rutinoside, and quercetin-3-rhamnoside identified flavonoid glycosides show that almost all of them contain either kaempferol, quercetin, or isorhamnetin core moieties. These indicate that the flavonoid core structures have sugar moieties attached to them. The sugar moiety is important in the bioavailability of flavonoid glycosides in humans. The total number of sugar

moieties, their position, and structure influences the antioxidant activity of such flavonoids [30].

Polyphenol metabolites such as flavonoids have been reported to possess various medicinal and pharmacological activities [31]. These properties are thought to be affected by the configuration structure of the flavonoid molecule. Therefore, it is not surprising that flavonoids such as quercetin have been utilised in clinical trials. High levels of quercetin and kaempferol in *Solanum lycopersicum* have been reported to exhibit antioxidant and pharmacological properties [32]. Flavonoids isolated from *Solanum torvum* have also been shown to have important antimicrobial activity. Flavonoids are thought to inhibit microbial growth through formation of complexes with bacterial cell walls [33]. Kaempferol, quercetin, and isorhamnetin aglycone are the most common flavonoids that are abundant in plant tissues [25].

3.9. Characterisation of Alkaloid Derivatives. The *S. retroflexum* methanol leaf extracts were found to contain two alkaloids. Molecules **29** and **30** with precursor ions at m/z 884 and 868 were identified as solasonine and solamargine, respectively (Table 1). The mass spectra of these molecules are shown in Figure 3. The difference in the m/z ratios of the

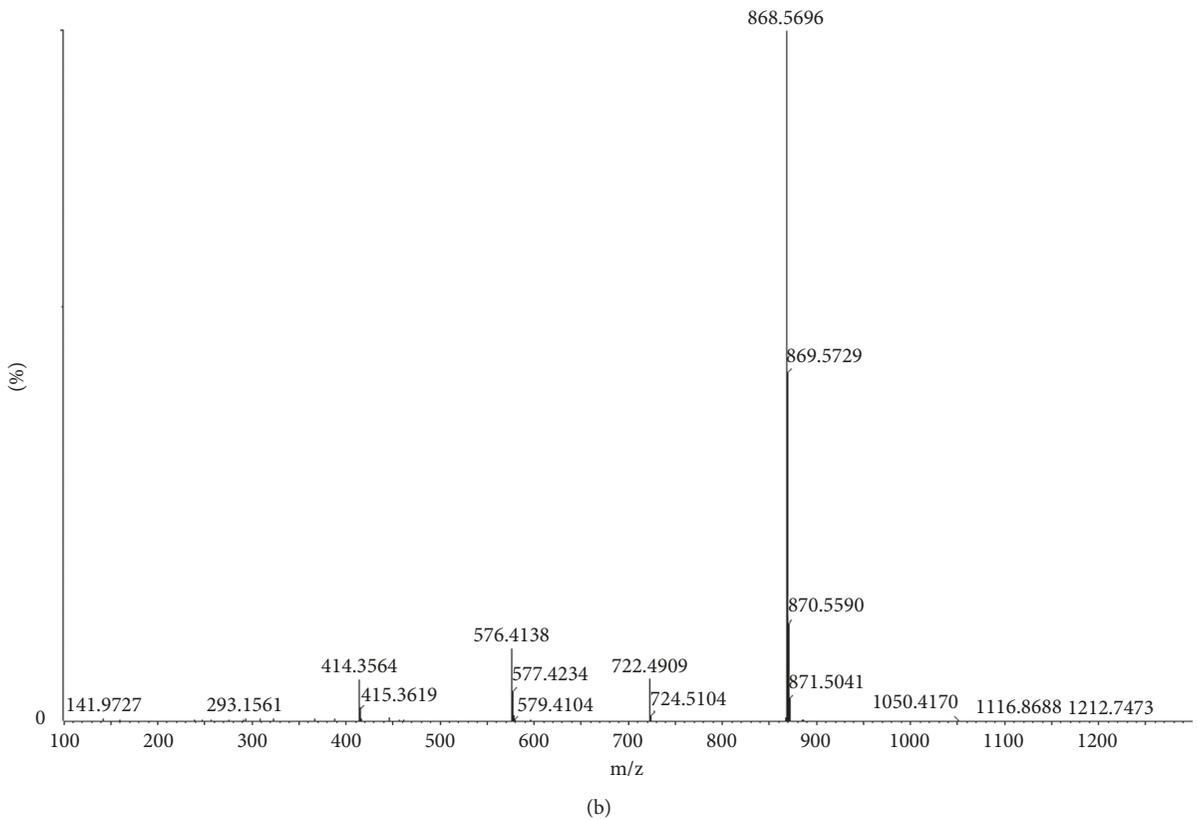
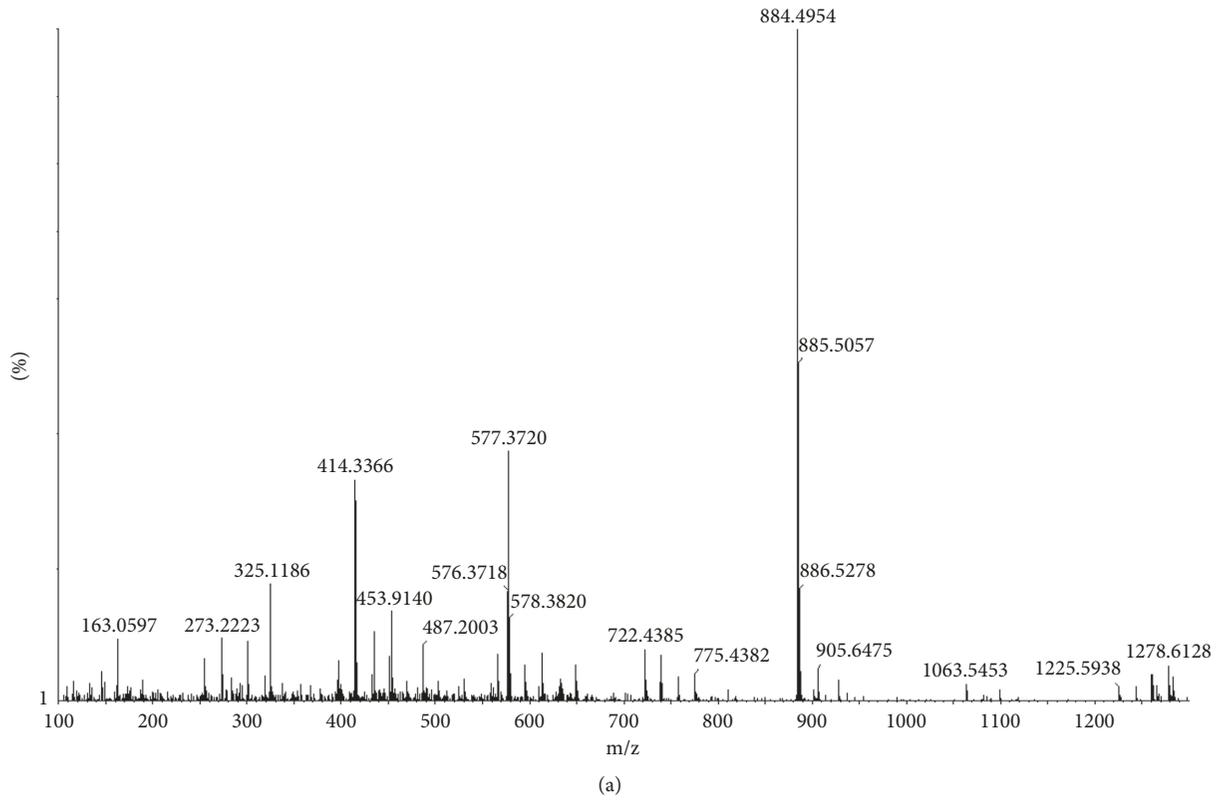


FIGURE 3: Mass spectra showing fragmentation pattern of solasonine (a) and solamargine (b).

identified Alkaloids is due to their aglycone variations. Despite this, their equivalent glycosides constitute either a solatriose or chacotriose as a sugar moiety. Thus, solasonine has

a molecular ion at m/z 884, and solamargine has one at m/z 868. In addition, the fragment ions at m/z 722, 576, and 141 were identical, and they elute very close to each other.

Glycoalkaloids (GAs) function chemically as defence compounds against herbivores, microorganisms, and competing plants. As a survival strategy, plants need to store sufficient amount of these defence compounds at strategic sites such as the bark, leaves, flower, seeds, or fruits [34]. Therefore, the presence of steroidal glycoalkaloid in *S. retroflexum* leaves should not be neglected because of its potential toxicity to humans. Solasonine and solamargine have also been identified in the leaves of *Solanum incanum* [35]. In their study, it was reported that the concentration of these alkaloids declined during the plants developmental stages with the least levels recorded at maturity. Other *Solanum* plants such as *Solanum nigrum* have been reported to produce solasonine and solamargine [36]. At low doses, the intake of glycoalkaloids may cause gastrointestinal disturbances such as vomiting, diarrhea, and abdominal pain, while at high doses, it may lead to serious complications such as rapid pulse and in some cases, coma and death [6].

4. Conclusions

According to our knowledge, this is the first comprehensive study on the phytochemical composition of *Solanum retroflexum* using the UHPLC-qTOF-MS method. A total of 30 different metabolites were identified with methanol extraction, and these include alkaloids, flavonoids, and cinnamic acid derivatives. The concentration of solvent did not affect the type of compounds extracted. The present study confirms the presence of phytochemical compounds in *S. retroflexum* similar to other *Solanum* plants.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Acknowledgments

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