Indian Journal of Natural Products and Resources
Vol. 14(3), September 2023, pp. 482-489
DOI: 10.56042/ijnpr.v14i3.5405

Characterisation and identification of phenolic compounds from *Asparagus laricinus* and *Senecio asperulus*

Polo-Ma-Abiele H. Mfengwana[1]*, Idah T. Manduna[2] and Samson S. Mashele[3]

[1]Department of Health Sciences, [2]Centre for Applied Food Safety and Biotechnology, [3]Centre for Quality of Health and Living, Central University of Technology, Free State, Private Bag X20539, Bloemfontein, South Africa

Received 04 October 2022; revised received 13 June 2023; accepted 28 June 2023

Nature has been a good supply of medicinal agents for thousands of years, and more than 50% of modern drugs are derived from natural sources such as plants. *Asparagus laricinus* and *Senecio asperulus* have been used in traditional remedies to treat inflammation-related disorders and cancer. This study aimed to determine the chemical composition of *A. laricinus* and *S. asperulus* crude extracts using Liquid chromatography-mass spectrometry (LC-MS/MS) based untargeted analysis. The LC-MS-based untargeted analysis showed the phytochemical profile of *A. laricinus* cladodes and *S. asperulus* roots, of which twenty-nine phytochemicals from different chemical classes were annotated, including organic acids, phenolic acids, flavonoids, and other unknown compounds. Both these plants are used as traditional medicines, and their pharmacological benefits are due to their phytochemical profile and high phenolic content.

Keywords: *Asparagus laricinus*, LC-MS analysis, Medicinal plants, Phytochemistry, *Senecio asperulus*

IPC code; Int. cl. (2021.01) – A61K 36/00, A61K 36/28, A61K 36/8965

Introduction

It is estimated that 61% of the 877 small molecule novel chemical entities that have been approved as medications globally during the previous 30 years originated from natural products[1]. About 75% of the drug discovered for non-communicable diseases consists of phenols from natural products. Phenolics are natural compounds obtained from plants which mainly function as a defence mechanism against predators[2]. Phenols also contribute to plant reproduction and plant-plant interference, as they are colourful attractants for birds and insects, thus helping seed dispersal and pollination[3]. Plants can produce chemical substances, mainly secondary metabolites, from which numerous compounds have been isolated. Secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, and phenolics are vital for humans and plants[4]. For instance, phenolic acids and flavonoids have various pharmacological activities when used by humans, including antioxidative, antimicrobial and anticarcinogenic effects[5-7].

Phenolic profiling studies are required as they can contribute to research on the isolation and synthesis of active compounds to help elucidate the therapeutic potential of medicinal plants. In the past, the identification of compounds was labour-intensive, time-consuming and required large sample quantities. However, modern drug discovery approaches apply full automation and robotics where hundreds of molecules can be screened using several assays within a short time and very small amounts of compounds[8]. Several techniques are used to identify and analyse plant's chemical constituents, such as Liquid Chromatography-Mass Spectrometry (LC-MS) and liquid chromatography–nuclear magnetic resonance spectroscopy. Liquid chromatography-mass spectrometry (LC-MS) is a chromatographic technique used to separate a mixture of compounds to characterise and identify the specific components of the mixture. Therefore, LC-MS combines MS and High-pressure liquid chromatography (HPLC) techniques, but not MS and LC. HPLC is an advanced type of Liquid chromatography where solvents travel under high pressures rather than through the force of gravity as with normal LC.

Furthermore, applying both techniques simultaneously reduces experimental error and improves accuracy[9]. Mass spectrometry in LC-MS helps to determine the elemental composition and

*Correspondent author
Email:pntsoeli@cut.ac.za

structural elucidation of a sample[10]. The elemental composition analysis is an informative technique used in mass spectrometry to identify unambiguous compounds. This study aimed to determine the chemical composition of crude extracts from *Asparagus laricinus* Burch. and *Senecio asperulus* DC using LC-MS coupled with tandem mass spectrometry.

Asparagus (Asparagaceae) species are known as medicinal plants with various biological properties[11]. The genus is rich in compounds of medicinal value, and previous investigations of *Asparagus* species identified 8-methoxy-5,6,4'-trihydroxyisoflavone-7-O-β-D-glucopyranoside, a novel compound from *Asparagus racemosus*, Oligofurostanosides *(curillins* G and H*)* and spirostanosides from *Asparagus curillins*, to mention a few[12]. The genus *Senecio* (Asteraceae) species are rich with essential oils containing epoxy furanoeremophilane and (E)-β-farnesene as their main compound[13]. *A. laricinus* is mostly used in Southern Africa to treat inflammation-related conditions and as a decoction for prostate cancer. Phytochemical screening of *A. laricinus* showed the presence of saponins, steroids, tannins, terpenoids and flavonoids[14]. *S. asperulus* is used to treat a variety of ailments in Lesotho and contains flavonoids, tannins, terpenoids, and steroids, as reported by Mfengwana *et al.*[15]. Both *A. laricinus* and *S. asperulus* are reported to have antioxidant, antibacterial and anticancer activity on prostate cancer cells and breast cancer cells[14-17]. The present study identifies and characterises their phenolic compounds as a step toward discovering bioactive entities.

Materials and Methods

Chemicals and reagents

Methanol, acetonitrile, 2-propanol, ammonium acetate and LC-grade formic acid were purchased from Sigma-Aldrich. Phenolic compound standards (protocatechuic acid, ellagic acid, syringic acid, gallic acid, ethyl gallate, gentisic acid, ferulic acid, myricetin 3-O-rhamnoside, quercetin 3-O-rhamnosyl-glucoside, quercetin 3-Ogalactoside, quercetin, catechin, epicatechin, epicatechin 3-O-gallate, gallocatechin, epigallocatechin, procyanidin dimer B2 and procyanidin trimer C1) with purity higher than 96% were purchased from Sigma Aldrich. Ultrapure water (18 MΩ cm−1) from the Milli-Q water purification system (Merck South Africa) was used. All other chemicals and reagents used in the experiments were of analytical grade.

Plant materials

The study received plant collection and export permits from the Ministry of Tourism Environment and Culture, Lesotho, and the Department of Economic Development, Tourism and Environmental Affairs (NC.553/2017), South Africa, for import approval. *S. asperulus* was collected from Mpharane, Mohale's Hoek district, Lesotho and *A. laricinus* was collected from Lakeview plots in Bloemfontein, South Africa. The collection was done during the summer season in January and February month. Both plants were authenticated by scientists at the National Botanical Gardens in Bloemfontein, South Africa, and allocated voucher numbers MAS001 for *A. laricinus*, and PHM01 for *S. asperulus*. Roots from *S. asperulus* DC*.,* and the cladodes from *A. laricinus* Burch. were separately washed, air-dried at room temperature (22°C), then ground into a fine powder using an electric blender and weighed. They were then stored in a cool place until analysis. The crude extracts were used in this study to imitate, as closely as possible, the concoctions frequently used for treating different ailments by local people.

Extraction method

The extraction was done using maceration. Plant material (10 g of the dried powdered roots and cladodes, respectively) were weighed, pulverised, and soaked separately in distilled water (DH_2O, methanol (MeOH), and dichloromethane (DCM), 50:50; v/v of methanol: dichloromethane (MeOH:DCM) and hexane, for 72 hours with occasional stirring using a mechanical shaker. The extracts were decanted, and a clean solvent was added to the plant material for further extraction until the solvents remained clear (this process was repeated three times). The extracts were then filtered, aqueous extracts were concentrated with a freeze-drier, and a rocket evaporator was used for the organic solvent extracts.

Untargeted analysis of phytochemicals by LC-MS/MS

LC-MS/MS analysis for the characterisation and identification of phenolic compounds from *S. asperulus* and *A. laricinus* crude extracts was carried out using the Waters Synapt G2, electrospray ionisation (ESI) positive/negative, Cone Voltage 15 V, lock mass: leucine encephalin instrument. This Agilent 1100 LC system consists of a degasser, binary pump, autosampler and column heater. The column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer with an ESI ion source. Data acquisition and mass spectrometric evaluation were done on a computer with an Acquity binary solvent manager instrument system. Waters BEH C18, 2.1 x 100 mm column was used for the chromatographic separation. The column was held at 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then it was kept for 4 min with 100% B; finally, the elution was achieved with a linear gradient from 100% B to 5% B for 2 min.

The flow rate was 0.4 mL/min, and the injection volume was 0.01 mL. The following parameters were used throughout all MS experiments: for electrospray ionisation with negative ion polarity, the capillary voltage was set to 3 kV, the drying temperature to 350°C and cone voltage of 15 V, the maximum nebuliser pressure to 15000 psi and the seal wash was 5 min. The total run time was 15 min, the scan speed was 26 000 m/z/s (ultra-scan mode), and the lock mass was Leucine enkephalin. The phenolics were identified using a combination of HPLC with diode array detection and liquid chromatography with atmospheric pressure chemical ionisation mass spectrometry (ESI-LC/MS/MS) based on their ultraviolet spectra, mass spectra and by comparison of the spectra with those of available authentic standards.

Compounds identification

Compounds were identified based on retention time and fragmentation pattern together with m/z values, which were obtained through LC-MS/MS analysis. Each extract component's relative retention times and mass spectra were compared with authentic samples (standards) and identified with the Elemental Composition database library programme. The obtained retention time and molecular mass of each peak observed were used to identify the molecular formula of each compound from elemental composition software. The mass spectra of the unknown compound obtained from sample extract by LC-MS were matched with mass spectra of the known compounds stored in the database of the National Institute Standard and Technology (NIST) library. Their structures were defined by the per cent similarity values. The name, molecular weight, molecular formula, and structure of the compounds were identified.

The major advantage of LC-MS includes sensitivity, specificity, and precision as analysis are performed at the molecular level[18]. Moreover, this instrument only requires small quantities of samples. It is very convenient for low or non-volatile organic compounds, which cannot be handled with other chromatographic techniques, such as gas chromatography.

Results

With LC-MS, compounds are separated based on their physical and chemical properties. Then, the components within each peak are detected by ionisation and identified based on their mass spectrum, as shown in Fig. 1. LC-MS coupled with tandem mass spectrometry of *A. laricinus* cladodes and *S. asperulus* roots yielded 41 phenolic compounds. These phenolic compounds were shown by peaks generated by the LC-MS/MS instrument, with molecular masses on the y-axis and retention time on the x-axis (Fig. 1-4). This study used the

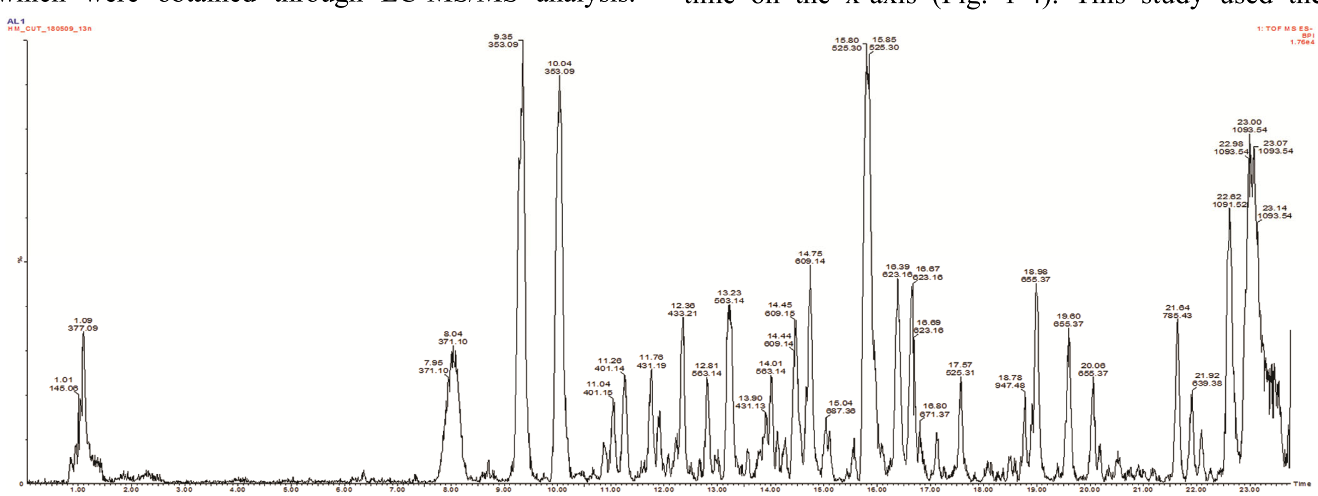

Fig. 1 — LC-MS/MS chromatogram of *Asparagus laricinus* methanol extract (ESI negative). Nineteen phenolic components were identified.

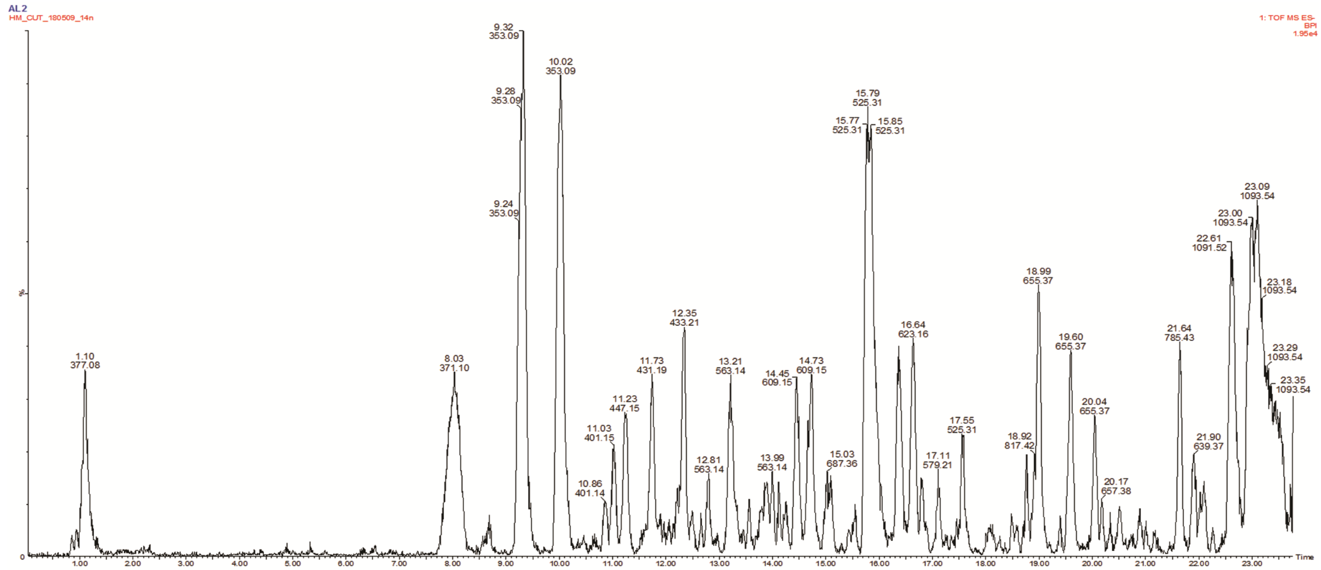

Fig. 2 — LC/MS chromatogram of *Asparagus laricinus* aqueous extracts (ESI negative). Twenty phenolic components were identified.

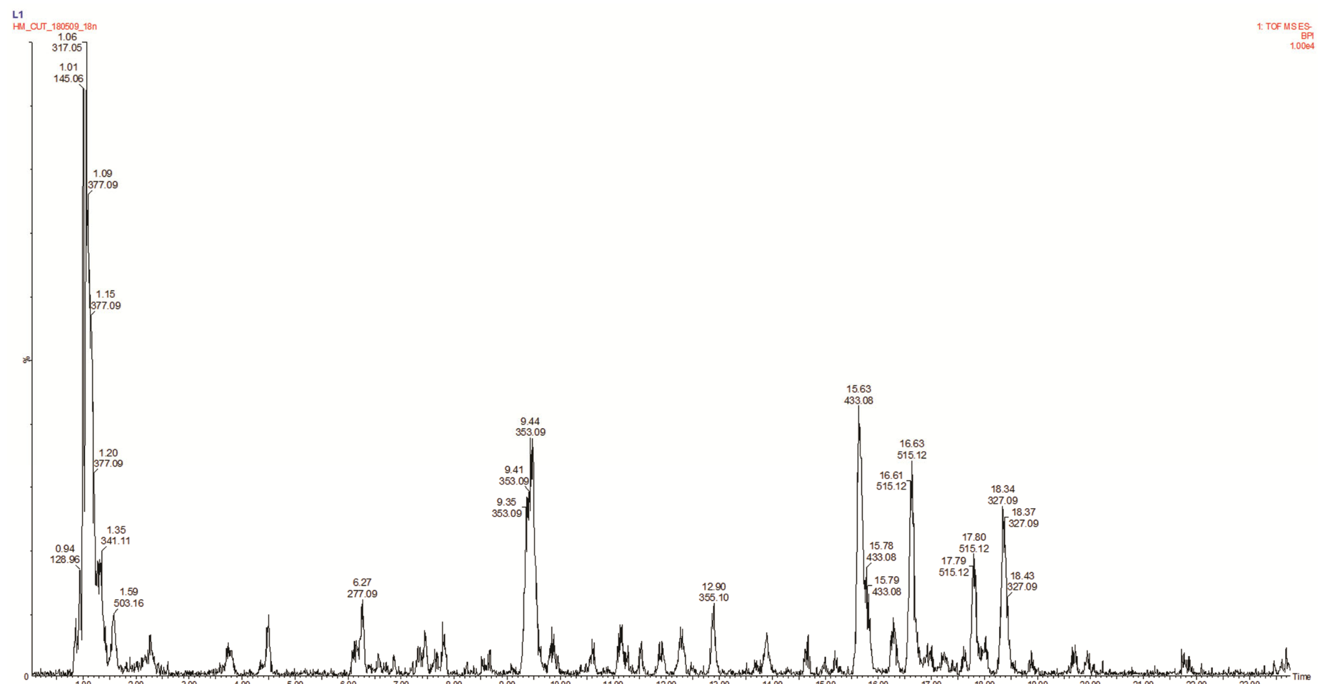

Fig. 3 — LC/MS chromatogram of *Senecio asperulus* methanol extracts (ESI negative). Thirteen phenolic components were identified.

deprotonated molecule mass (m/z) measured by the MS in ESI negative and positive mode to determine the molecular formula of the compounds extracted. Fragmentation of the compounds by the mass spectrum was also considered to obtain the molecular chemical formula of the compounds that formed significant peaks reported in Tables 1 and 2.

As shown in Fig. 1, LC-MS analysis of the methanol extract of *A. laricinus* resulted in the identification of nineteen compounds. The first identified compound had a molecular mass of 593,2 and a retention time of 11,92. It had more than 90% similarity with the standard mass spectra in the library. The LC-MS analysis of *S. asperulus* DC.

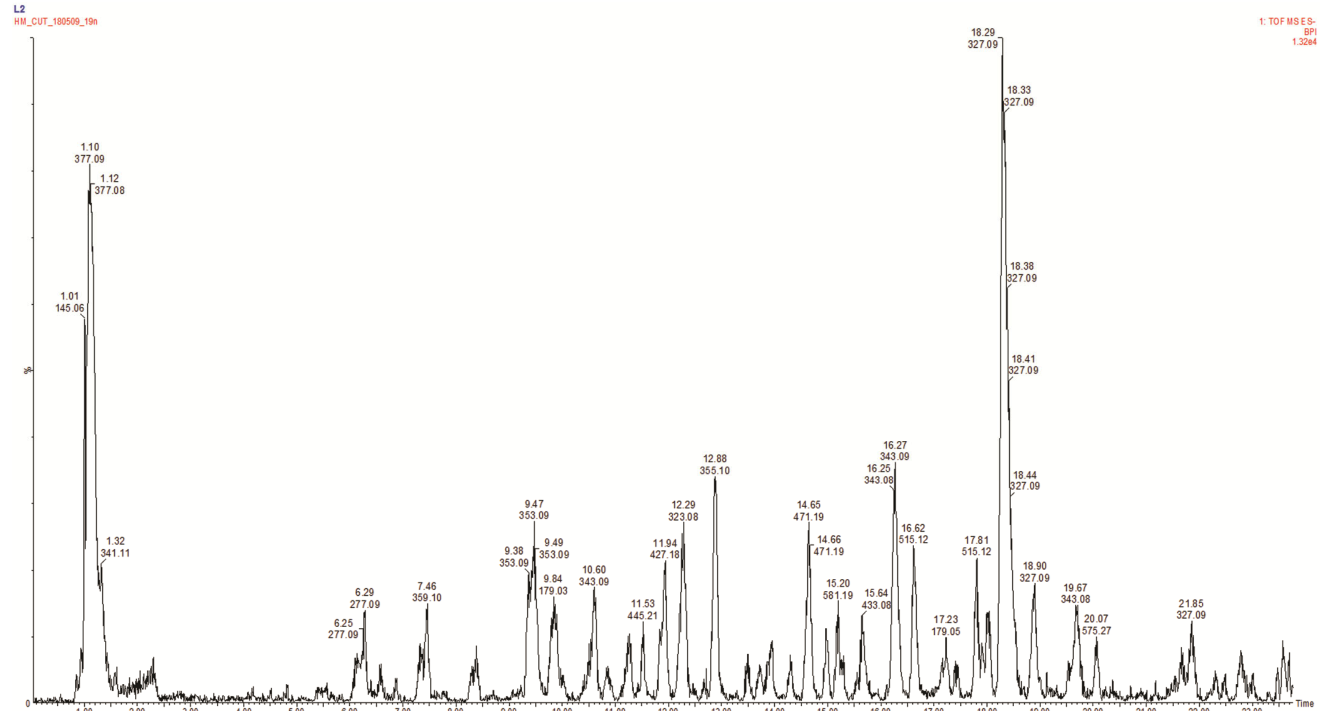

Fig. 4 — LC/MS chromatogram of *Senecio asperulus* aqueous extracts (ESI negative). Twenty-four phenolic components were identified.

Table 1 — Summary of the most abundant compounds present in *Asparagus laricinus* Burch. cladodes. (m/z) = Molecular mass, Rt = retention time, ID= identification.

	Rt	m/z [M-H]-	Formula [M-H]-	MS/MS Fragments	UV max	ID	Phytochemical class
1	8,04	371,1	C16H19O10	281 251 197 167	289	Unknown	Not known
2	9,35	353,08	C16H17O9	233 205,05	294	Unknown	Not known
3	10	353,08	C16H17O9	263 233 205 161	296	Unknown	Not known
4	10,89	755,2	C33H39O20	593 401 284 193 161	263	Unknown	Not known
5	11,26	401,14	C18H25O10	269 161 113 101	288	Unknown	Not known
6	11,8	519,17	C22H31O14	459 431 385	341	Unknown	Not known
7	11,92	593,2	C27H29O15	473 383 353 297	270 332	Luteolin 7-O-rutinoside	Flavonoids
8	12,36	433,2	C20H33O10	399 285 225 145 119	310	Unknown	Not known
9	12,67	563,14	C26H27O14	383 353 297 175	330	Apigenin arabinoside-glucoside	Flavonoids
10	13,21	563,14	C26H27O14	473 443 383 353 297	333, 271	Apigenin galactoside-arabinoside	Flavonoids
11	13,8	431,13	C22H23O9	339 295 267 241 226	279	3-Methoxynobiletin	Flavonoids
12	13,99	563,14	C26H27O14	473 443 383 353 297	332	Apigenin 7-O-apiosyl-glucoside	Flavonoids
13	14,1	787,27	C28H51O25	579 417 361 181	271	Unknown	Not known
14	14,24	461,14	C23H25O10	417 269 271 256	280	Unknown	Not known
15	14,47	609,14	C27H29O16	300 271 245 203 151	348	Quercetin 3-O-rhamnosyl-galactoside	Flavonoids
16	14,7	609,14	C27H29O16	300 271 245 203 151	348	Rutin (Quercetin 3-O-rhamnosyl-glucoside)	Flavonoids
17	15,03	641,35	C33H53O12	609 479 447 429 225	320	Unknown	Not known
18	15,54	593,15	C27H29O15	285 255 227 193	264	Kaempferol 3-O-galactoside 7-O-rhamnoside	Flavonoids
19	15,8	479,3	C27H43O7	479 159	247	Ecdysterone	Steroids
20	16,4	623,16	C28H31O16	314 299 271	351	Isorhamnetin 3-O-glucoside 7-O-rhamnoside	Flavonoids
21	16,6	623,16	C28H31O16	315 300 255	352	Unknown	Not known
22	17,12	417,15	C22H25O8	771 579 477 417 314 000	347	Syringaresinol	Polyphenols
23	17,57	479,3	C27H43O7	319 285 165 159 119	280	20-hydroxyecdysone	Steroids

Table 2 — Summary of most abundant compounds present in *Senecio asperulus* DC. roots. (m/z) = Molecular mass, Rt = retention time, ID= identification

No.	RT	m/z [M-H]-	Formula [M-H]-	MS/MS Fragments	UV max	ID
1	9,45	353,088	C16H17O9	191	325	chlorogenic acid isomer
2	11	353,0876	C16H17O9	191	305	chlorogenic acid isomer
3	15,6	433,0771	C20H17O11	271,179,135,253	330	Unknown
4	16,6	515,1187	C25H23O12	135,179,191,353	326	dicaffeoylquinic acid
5	17,8	515,1198	C25H23O12	173,179,191,135,353	326	dicaffeoylquinic acid
6	18,4	327,088	C18H15O6	97	280	Unknown

extract resulted in identifying two compounds with more than 90% similarity with the standard mass spectra in the library. Twenty-three compounds were found to be the most abundant compounds in the cladodes of *A. laricinus,* with twelve known and eleven unknown compounds (Table 1). Flavonoids comprised 75% of the known compounds; the rest comprised two steroids and a polyphenol. Four compounds were identified, while two were unknown from the six compounds found to be most abundant in *S. asperulus.*

Discussion

The LC-MS/MS technique was used to characterise and identify the phenolic content of *A. laricinus.* and *S. asperulus* plant extracts. A total of 41 compounds, of which 25 compounds were discovered from *A. laricinus* and *S. asperulus* using both the negative and the positive ESI modes. However, only 23 compounds from *A. laricinus* and six compounds from *S. asperulus* were considered abundant and reported in this study. The abundance of compounds characterised and identified from these plants correlated with the reported total phenolic content reported from previous studies that indicate that *A. laricinus* has more phenols than *S. asperous*. Previous phytochemical screening performed on *S. asperulus* revealed the presence of glycosides from the aqueous extract of a mixture of leaves and roots. The methanol and acetone extracts had flavonoids, while phytosterols were found in acetone extracts only[19]. In the present study, *S. asperulus* had two known compounds (a chlorogenic acid isomer and a dicaffeoylquinic acid) and nineteen unknown compounds. The present study is the first report of the presence of these compounds from *S. asperulus* collected from Lesotho. Both chlorogenic acid isomer and dicaffeoylquinic acid have been reported to have antioxidant potential due to their ability to scavenge free radicals[20–21]. Therefore, the presence of this molecule in *S. asperulus* supports its activity against free radicals previously reported by Mfengwana *et al.*[15].

The conclusive accomplishment of isolating and synthesising bioactive plant constituents depends on selecting the correct plant part containing the active compounds and characterising the phenolics present. This is because secondary metabolites are expressed in various combinations and concentrations in different parts of the plant; for example, the roots of the plants may contain fewer phenols than leaves. Consequently, certain secondary metabolites in specific organs and variations in bioactivity are often encountered in different parts of the same plant[22]. This study focused on the plant parts used in traditional medicine (roots and cladodes of *S. asperulus* and *A. laricinus,* respectively) as they were expected to contain high concentrations of the medicinally active phytochemicals. Fuku *et al.*[23], isolated and identified three compounds using GC/MS; indole-3-carbinol, α-sitosterol, and ferulic acid from the *A. laricinus* roots, while this study revealed more phenolics from the cladodes of this plant. This supports the distribution of secondary metabolite variations between different parts of the same plant, as LC/MS and GC/MS have similar sensitivity in detecting metabolites[24,25].

The following compounds were identified from *A. laricinus* Burch. cladodes: Luteolin 7-O-rutinoside, Apigenin arabinoside-glucoside, Apigenin galactoside-arabinoside, 3-Methoxynobiletin, Apigenin 7-O-apiosyl-glucoside, Quercetin 3-O-rhamnosyl-galactoside, Rutin (Quercetin 3-O-rhamnosyl-glucoside), Kaempferol 3-O-galactoside 7-O-rhamnoside, Ecdysterone, Isorhamnetin 3-O-glucoside 7-O-rhamnoside, Syringaresinol, and 20-hydroxyecdysone. This is the first report on identifying these compounds from *A. laricinus*; however, some of the identified flavonoids have been reported in the Asparagaceae family. Steroidal saponins have been highlighted as major bioactive constituents of the Asparagus family, and the primary chemical constituents of *Asparagus* species are tannins, flavonoids such as kaempferol, quercetin, and rutin, and their leaves contain diosgenin and quercetin-3-glucuronide[12].

A. laricinus cladodes were found to have flavonoids in abundance (Table 1). Flavonoids are classified into various classes[26], such as flavonols (quercetin), flavones (apigenin), flavanones (hesperetin, naringenin), flavonoid glycosides (astragalin, rutin), flavonolignans (silibinin), flavans (catechin, epicatechin) to name a few. Flavonoids are reported as antioxidant, antiproliferative, anti-tumour, antimicrobial, estrogenic, acetylcholinesterase, and anti-inflammatory agents. They are also used to treat cancer, cardiovascular disease and neurodegenerative disorders[27]. The abundance of flavonoids found in this study supports the anticancer, antioxidant, antimicrobial and anti-inflammatory activity yielded by *A. laricinus*[14,17], which indicates a correlation between the identified active compounds and pharmacological activities of the plant. To further elucidate chemical structures and the action of each active compound in *A. laricinus* cladodes and *S. asperulus* DC. roots, each identified compound - even the unknown ones- must be isolated and analysed. The pharmacological activities of present phenols can be investigated further with isolated compounds to demonstrate whether these phenolic compounds function in synergy or independently. This will enable isolating pure active compounds and synthesizing novel anticancer compounds.

Conclusion

Traditional medicinal plants have been a good source of new medicinal agents for thousands of years, and more than 50% of modern drugs have been isolated from plants. The LC-MS analysis of extracts from *A. laricinus* Burch. and *S. asperulus* DC. demonstrated the presence of twenty-nine compounds, of which only fourteen were identified. The rest are unknown and thus still need to be investigated. *A. laricinus* had twelve known compounds: Luteolin 7-O-rutinoside, Apigenin arabinoside-glucoside, Apigenin galactoside-arabinoside, 3-Methoxynobiletin, Apigenin 7-O-apiosyl-glucoside, Quercetin 3-O-rhamnosyl-galactoside, Rutin (Quercetin 3-O-rhamnosyl-glucoside), Kaempferol 3-O-galactoside 7-O-rhamnoside, Ecdysterone, Isorhamnetin 3-O-glucoside 7-O-rhamnoside, Syringaresinol and 20-hydroxyecdysone. Two known compounds, chlorogenic acid isomers, dicaffeoylquinic acid were found in *S. asperulus*. The identified compounds have pharmacological properties that may benefit human beings as chemopreventative agents. However, this can only be validated after these active compounds' isolation and structural elucidation using High-performance liquid chromatography and nuclear magnetic resonance (NMR).

References

1 Calixto J B, The role of natural products in modern drug discovery, *An Acad Bras Cienc*, 2019, **91**, e20190105.

2 Velderrain-Rodríguez G, Palafox-Carlos H, Wall-Medrano A, Ayala-zavalaJ, Chen C O, *et al*., Phenolic compounds: their journey after intake, *Food Funct*, 2014, **5**(2), 189-197.

3 Oksana S, Marian B, Mahendra R and Hong B S, Plant phenolic compounds for food, pharmaceutical and cosmetics production, *J Med Plant Res*, 2012, **6**(13), 2526-2539.

4 Rafael C D, Magda N L and Nadia R, Quantification of phenolic constituents and antioxidant activity of *Pterodonemarginatus* vogel seeds, *Int J Mol Sci*, 2008, **9**, 606-614.

5 Noratto G D, Bertoldi M C, Krenek K, Talcott S T, Stringheta P C, *et al*., Anticarcinogenic effects of polyphenolics from Mango (*Mangifera indica*) varieties, *J Agric Food Chem*, 2010, **58**(7), 4104-4112.

6 Pierson J T, Curry M C, Shaw P N, Dietzgen R G, Gidley M J, *et al*., Polyphenolic contents and the effects of methanol extracts from mango varieties on breast cancer cells, *Food Sci Biotechnol*, 2015, **24**(1), 265-271.

7 Wang W, Guo J, Zhang J, Peng J, Liu T, *et al*., Isolation, identification and antioxidant activity of bound phenolic compounds present in rice bran, *Food Chem*, 2015, **171**, 40-49.

8 Peixoto Araujoa N M, Arrudaa H S, Neves dos Santos F, Rodrigues de Morais D, Pereira G A, *et al*., LC-MS/MS screening and identification of bioactive compounds in leaves, pulp and seed from *Eugenia calycina* Cambess, *Food Res Int*, 2020, **137**, 109556.

9 Lim C K, and Lord G, Current development in LC-MS for pharmaceutical analysis, *Bio Pharm Bull*, 2002, **25**(5), 547-557.

10 Pitt J J, Principles and applications of Liquid Chromatography-Mass Spectrometry in clinical biochemistry, *Clin Biochem Rev*, 2009, **30**(1), 19-34.

11 Saxena V K and Chaurasia S, A new isoflavone from the roots of *Asparagus racemosus*, *Fitoterapia*, 2001, **72**, 307–309.

12 Negi J S, Singh P, Joshi G P, Rawat M S and Bisht V K, Chemical constituents of Asparagus, *Pharmacogn Rev*, 2010, **4**(8), 215–220.

13 Kahriman N, Tosun G, Terzioğlu S, Karaoğlu S A and Yayl N, Chemical composition and antimicrobial activity of the essential oils from the flower, leaf, and stem of *Senecio pandurifolius*, *Rec Nat Prod*, 2011, **5**(2), 82-91.

14 Ntsoelinyane P H and Mashele S S, Phytochemical screening, antibacterial and antioxidant activities of

Asparagus laricinus leaf and stem extracts, *Bangladesh J Pharmacol*, 2014, **9**, 10-14.

15 Mfengwana P H, Mashele S S and Manduna I T, *In vitro* antibacterial, antioxidant and anti-inflammatory effects of *Senecio asperulus* and *Gunnera perpensa* from Mohale's Hoek, Lesotho, *Pharmacogn J*, 2019, **11**(4), 730-739.

16 Mashele S S and Kolesnikova N, *In vitro* anticancer screening of *Asparagus laricinus* extracts, *Pharmacologyonline*, 2010, **2**, 246–252.

17 Mfengwana P H, Mashele S S and Manduna I T, Cytotoxicity and cell cycle analysis of *Asparagus laricinus* Burch. and *Senecio asperulus* DC. on breast and prostate cancer cell lines, *Heliyon*, 2019, **5**(5), e01666.

18 Mugomeri E, Chatanga P, Hlapisi S and Rahlao L, Phytochemical characterisation of selected herbal products in Lesotho, *Lesotho Med Assoc J*, 2014, **12**, 38–47.

19 Parasuraman S, Anish R, Balamurugan S, Muralidharan S, Kumar K J, *et al.*, An overview of liquidchromatography-mass spectroscopy instrumentation, *Pharm Methods*, 2014, **5**(2), 47-55.

20 Ortal D, Hugo E G, Shlomo G and Margalit B, Antioxidant activity of 1,3-dicaffeoylquinic acid isolated from *Inula viscosa*, *Food Res Int*, 2009, **42**(9), 1273-1280.

21 Xu J G, Hu Q P and Liu Y, Antioxidant and DNA-protective activities of chlorogenic acid isomers, *J Agric Food Chem*, 2012, **60**(46), 11625-11630.

22 Szwajkowska-Michałek L, Przybylska-Balcerek A, Rogozi ´nski T and Stuper-Szablewska K, Phenolic compounds in trees and shrubs of central Europe, *Appl Sci*, 2020, **10**, 6907.

23 Fuku S, Al-Azzawi A M, Madamombe-Manduna I T and Mashele S, Phytochemistry and free radical scavenging activity of *Asparagus laricinus*, *Int J Pharmacol*, 2013, **9**(5), 312-317.

24 Marquet P, LC-MS vs. GC-MS, online extraction systems, advantages of technology for drug screening assays, *Methods Mol Biol*, 2012, **902**, 15-27.

25 Perez E R, Knapp J A, Horn C K, Stillman S L, Evans J E, *et al.*, Comparison of LC–MS-MS and GC–MS analysis of benzodiazepine compounds included in the drug demand reduction urinalysis program, *J Anal Toxicol*, 2016, **40**, 201-207.

26 Singh M, Kaur M and Silakari O, Flavones: An important scaffold for medicinal chemistry, *Eur J Med Chem*, 2014, **84**, 206-239.

27 Cushnie T P T and Lamb A J, Antimicrobial activity of flavonoids, *Int J Antimicrob Agents*, 2005, **26**, 343-356.