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UPLC–QTOF/MS tentative identification of phytochemicals from *Vernonia amygdalina* Delile acetone and ethanol leaf extracts

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Background: *Vernonia amygdalina* Delile is a woody medicinal plant that is commonly used in various folklore medicine in South Africa against helminthic, protozoal and bacterial infections. Despite its high medicinal values, the chemical profile of this plant has not been fully elucidated and remains unclassified.

Aim: The present study reports the tentative identification of untargeted metabolite profiling from *V. amygdalina* Delile acetone and ethanol leaf extracts.

Setting: The study took place in the Nkomazi municipality which is located in the eastern part of the Ehlazeni district municipality of the Mpumalanga province.

Methods: Metabolites were identified using ultraperformance liquid chromatography (UPLC) method coupled with quadrupole time of flight mass spectrometry (qTOF/MS).

Results: The UPLC-qTOF-MS analysis tentatively revealed the presence of prominent metabolites, 17 of which were identified in acetone extracts and 12 in the ethanol extracts of *V. amygdalina* Delile. The identified metabolites included compound classes such as terpenoids which were highest in the acetone extracts (23.53%) and flavonoids which were highest in the ethanol extracts (25%).

Conclusion: The findings reveal several significant bioactive compounds which may contribute to the development of new antifungal drugs.

Contribution: The results showed significant chemical differences between both extracts of the plant in the profiles. The findings revealed several significant bioactive compounds which may contribute to the development of new antifungal drugs.

Keywords: Compounds; UPLC; metabolites; sesquiterpenes; phytochemicals.

Introduction

Medicinal plants are vital sources of medicine for both traditional and conventional medicinal purposes around the world. They perform critical roles in the majority of developing countries, as they provide basic healthcare to roughly 80% of the population (Fullas 2007; Maroyi 2013). Secondary metabolites are found in the majority of medicinal plants and are thought to be the driving force behind plant chemical defence activities in response to stress, diseases and predators (Otang-Mbeng & Sagbo 2019). Secondary metabolites in plants comprise a wide range of phytochemicals such as alkaloids, phenolics, terpenoids and their derivatives found in plant extracts. They are thought to confer an evolutionary benefit to plant species by being activated in response to biotic and abiotic stress (Kennedy & Wightman 2011). This evolutionary metabolite defence system has produced bioactive chemicals with potential use in medicine, pharmacy and biotechnology. An increasing number of novel compounds from several plant species are constantly being identified and investigated for their potential pharmacological use (Joseph & Priya 2011). Medicinal plant extracts have been used for decades for their antimicrobial purposes including antifungal activity (Ben-Shabat et al. 2020). The abundance of chemical components found in medicinal plants gives a large room for the discovery of new organic antifungal agents (Parekh & Chanda 2007). According to Basile et al. (2000), several studies have identified active medicinal plant compounds such as flindersine (Duraipandiyan & Ignacimuthu 2009), dasymaroine A (Yu et al. 2019) and rutin (Al-

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Majmaie et al. 2019), which are active against pathogens such as *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*, respectively. While effective compounds have been developed from traditional medicinal plant remedies with proven activity against antibiotic-resistant strains of bacteria (Koné et al. 2004), there is still a need to identify new antimicrobial compounds with complex chemical structures and novel mechanisms of action that are active against plant pathogens. Because of their antifungal properties and manageable side effects, medicinal plant extracts may be a better alternative for the development of active chemicals against various diseases in several agricultural crops.

Vernonia amygdalina Delile is a woody shrub that grows up to 10 m and is the most largely cultivated species from over 1000 species of the *Vernonia* genus (Clement et al. 2014; Toyang & Verpoorte 2013). *Vernonia amygdalina* Delile is the most studied African species within the Asteraceae family (Farombi & Owoeye 2011). The plant has petiolated leaves with a bitter taste, hence the name 'bitter leaf'. According to Alara et al. (2017), the bitterness of the plant leaves is linked to the presence of alkaloids, tannins, saponins and glycosides. This plant exhibits antioxidant properties due to the bioavailability of phenolic acids contained in plant extracts (Konaté et al. 2015). The presence of these bioactive compounds gives this plant medicinal properties. The reports have revealed that *V. amygdalina* Delile has been previously studied and screened intensively for antimicrobial, antioxidant, antibacterial, cathartic and anticancer activity, among others (Owoeye et al. 2010).

Investigating natural products with ultraperformance liquid chromatography (UPLC) coupled with quadrupole time-of-flight mass spectrometry (QTOF/MS) offers effective separation and good sensitivity. The UPLC has the ability to identify fragmentation processes of metabolites by employing newer mass spectrometry $^{\text{Elevated energy}}$ (MS^E) methods to acquire tandem mass spectrometry (MS/MS) (without specific precursor ion selection) data at both low and high energy from a single injection (Konishi et al. 2007; Nordström et al. 2006; Wrona et al. 2005). Therefore, UPLC–QTOF/MS^E is a very versatile technique and is increasingly important in metabolomics (Zhao & Lin 2014). Even though *V. amygdalina* Delile has a high medicinal value with a huge pool of bioactive chemicals, the metabolite profiling of this plant from South Africa remains not fully elucidated and unclassified. As a result, this work seeks to profile bioactive secondary metabolites from the *V. amygdalina* Delile acetone and ethanol leaf extracts using the UPLC–QTOF/MS method.

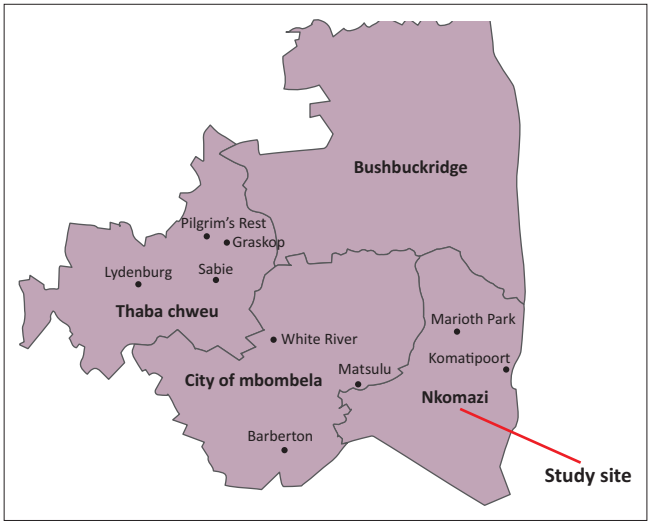
Research methods and design

Plant collection, identification and extraction

The leaves of *V. amygdalina* Delile were collected in April 2021 from the Nkomazi region (25°44.946′ S, 31°32.49′ E) of the Mpumalanga province, South Africa (Figure 1). Plant specimens were identified, and voucher specimens (CM001) of *V. amygdalina* Delile were prepared and deposited in the South African National Biodiversity Institute (SANBI) herbarium in Pretoria. The leaves of *V. amygdalina* Delile were detached from the rest of the plant and washed with clean tap water to remove debris. The leaves were further washed with distilled water and were then oven dried to a constant weight at 40 °C for 72 h. The leaves were pulverised to a homogeneous powder using a sterile electric blender (Commercial Blender type GB27, Hamilton Beach Brands, Inc. China). The powdered samples were stored in airtight containers to preserve the biomolecules present in the plant and stored at room temperature. The crude plant extract was prepared following the Soxhlet extraction method as described by Redfern et al. (2014). Approximately 50 g of the powdered plant was extracted separately in 300 mL of 70% ethanol and acetone (99.99%) on an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 h. The use of the two solvents was influenced by their availability. The extracts were thereafter filtered using a Buchner funnel and Whatman No. 1 filter paper, and then the filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH and Co, Germany) at 40 °C (Otang-Mbeng et al. 2012). Each extract was exposed to fan air for solidification (Koduru, Grierson & Afolayan 2006).

Ultraperformance liquid chromatography–quadrupole time-of-flight mass spectrometry profiling

A 0.22-µm polytetrafluoroethylene filter was used to filter the supernatants. A Quadrupole 120 time-of-flight (QTOF) mass spectrometer UPLC–QTOF/MS (Waters, Milford, Massachusetts, United States) was used to identify and quantify predominant secondary metabolites. An ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm i.d., 1.7 µm × 10–6 m; Waters) was used for all analyses. The mobile phase was composed of acetonitrile (A) and 0.1% formic acid, v/v (B), with the following gradient elution: 0–8 min, 95% – 80% A; 8–12 min, 80% – 70% A; 12–15 min, 70–65 A; 15–18 min, 65% A; 18–21 min, 65% – 20% A; 21–23 min, 20–5% A; 23–24 min, 5% A and 25–30 min, 95% A. The flow rate of the mobile phase was



Source: From Mashile, S., Tshisikhawe, M. & Masevhe, N., 2019, 'Indigenous fruit plants species of the Mapulana of Ehlanzeni district in Mpumalanga province, South Africa', *South African Journal of Botany* 122, 180–183. https://doi.org/10.1016/j.sajb.2018.09.031

FIGURE 1: Ehlanzeni local municipalities map.

0.4 mL/min and the temperatures of the column and auto-sampler were maintained at 30 °C and 10 °C, respectively. Data were analysed in both negative and positive ionisation modes. Data were processed using MS-DIAL and MS-FINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) (Liu et al. 2018; Tsugawa et al. 2018). Functions 1 (unfragmented channel) and 2 (fragmented channel) of the Waters MSe data were processed by MS-DIAL to produce MS1 and MS2 spectra as well as extracted ion chromatograms with associated peak height intensity data. Since calibration standards are not available for most of these compounds, the peak height intensity was converted to concentration in a semiquantitative manner by interpolation of a calibration curve for catechin acquired under the same instrumental conditions. Each deconvoluted feature (alignment in MS-DIAL), together with its associated MS1 and MS spectra, was exported from MS-DIAL to MS-FINDER. Based on the accurate mass elemental compositions, possible compounds were identified from the listed databases and then subjected to *in silico* fragmentation. According to the spectral match between the *in silico* and measured spectra, a score (out of 10) is assigned to each of the possible compound matches, with the highest score being accepted as the most likely (assuming a score of at least 4).

Data analysis

Using MarkerLynx version 4.1 (Waters, Milford, Massachusetts, United States), alignment and peak detection and raw data filtering were conducted. A mass range of 100 Da – 1000 Da, 5–21-min retention time and 50 mDa tolerance time were used as parameters. In addition, 0.4-min retention time tolerance, a 500-intensity threshold/counts of collection parameters and a noise elimination level of 1.00 were all set. SIMCA-P+ (13.0) software (Umetrics, Umeå, Sweden) was used to determine m/z data pair and retention time for each peak.

Results and discussion

Ultraperformance liquid chromatography–quadrupole time-of-flight–mass spectrometry identification and characterisation of the bioactive metabolites

A total of 17 bioactive metabolites were detected between 4.5 and 16.01 min in the acetone leaf extracts of *V. amygdalina* Delile, and a total of 12 metabolites between 4.13 and 14.64 min in ethanol leaf extract (Table 1) of *V. amygdalina* Delile in both negative and positive ionisation mode were detected. Compounds were tentatively identified according to their molecular formula, retention time and their fragment ions in comparison with data from the literature. The chromatograms displaying the peaks in Table 1 are shown in Figure 2. As shown in Table 2, identified metabolites classes included terpenes (23.52%), alkaloids (5.88%), sesquiterpenes (17.64%), lipids (11.76%), glycosides (5.88%), steroids (5.88%), flavonoids (5.88%) and an unidentified class (23.52%) in the acetone leaf extract. The ethanol leaf extracts are composed of terpenes (8.33%), fatty acids (33.33%), flavonoids (25%), saponins (16.67%) and an unidentified class of metabolites (16.67%) (Table 2). The current investigation found that acetone was more effective in extracting compounds, and hence it produced more metabolites than the ethanol extract. Eloff, Masoko and Picard (2007) previously described acetone as a safe and effective extractant. This is because of its volatility, miscibility with polar and nonpolar solvents and low toxicity to plant material tested (Mahlo, McGaw & Eloff 2010), unlike ethanol, which can only remove polar compounds (Eloff et al. 2007).

Terpenes

The terpenes identified in the acetone leaf extract of this study included thiamine, ascorbic acid, 10-O-methyl-alismoxide and aspersteroid A (peaks 7, 10, 12 and 13, respectively). However, salvic acid (peak 10) is the only terpene identified in the ethanol leaf extract of *V. amygdalina* Delile in this study.

Terpenes are well known for their chemical defence properties in plants, and their presence in the selected plant in this study serves as a good potential tool for fungal disease control, as recommended by Zacchino et al. (2017). Peak 10, characterised as salvic acid giving ion mass 322.2499 m/z at 13.5 min (Figure 3), has been discovered to possess potent antifungal properties against *Botrytis cinerea*, a phytopathogenic fungus that damages the flowers, fruits, leaves and also stems of over 200 plant species (Wang et al. 2021). Okoi et al. (2015) discovered that ethanol extracts of *V. amygdalina* Delile were effective at inhibiting the postharvest fungal diseases *Rhizopus stolonifer* and *Fusarium moniliforme*.

Flavonoids

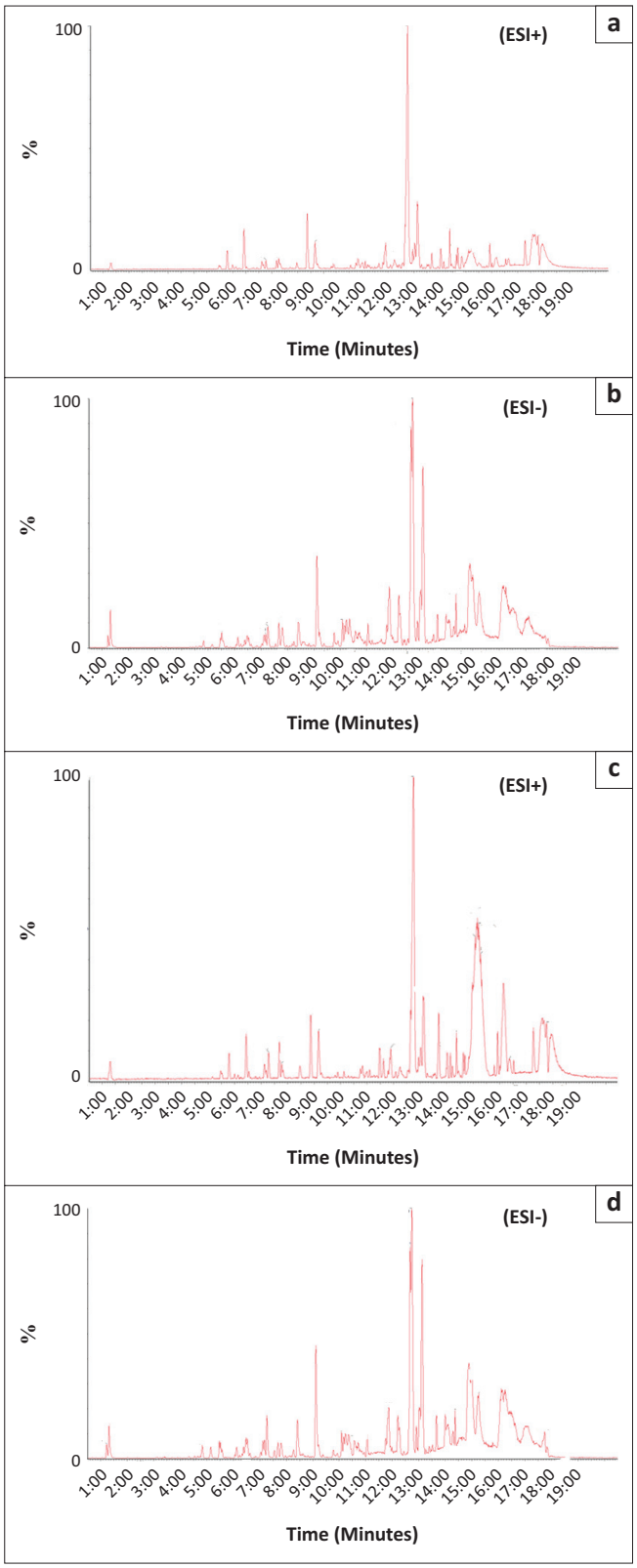
As shown in Table 1, UPLC–QTOF/MS analysis allowed for the detection and identification of three quercetin derivatives such as luteolin, quercetin and quercetin deoxyhexoside (peaks 1, 2 and 4, respectively) in ethanol leaf extracts of *V. amygdalina* Delile. In contrast with terpenes, the results indicate that more flavonoid compounds were extracted from *V. amygdalina* Delile leaves extracted with ethanol than acetone. Flavonoids remain an attraction to many researchers because of their wide range of reported pharmacological activities. This chemically diverse group is synthesised by plants in response to microbial infections; hence, most of them, such as quercetin (Figure 4), have been reported to be potent antifungal agents against fungal strains such as *Cryptococcus neoformans* (Górniak, Bartoszewski & Króliczewski 2019; Lunardelli Negreiros de Carvalho et al. 2016). The antioxidant, anti-inflammatory and antimicrobial role of flavonoids are well reported in the literature. In a different study by Tomita-Yokotani et al. (2003), quercetin inhibited the growth of *Arabidopsis thaliana* and *Neurospora crassa*.

Sesquiterpenes

According to Howard, Izevbigie and Opata (2006), *V. amygdalina* Delile contains bioactive sesquiterpene lactones with potent antifungal properties. On the other hand, some of the chemical constituents that appear in lower amounts in this extract, such as terpenoids, steroids and

TABLE 1: Tentative identification of secondary metabolites present in acetone and ethanol leaf extract of *V. amygdalina* Delile using ultraperformance liquid chromatography– quadrupole time-of-flight–tandem mass spectrometry analysis in electrospray ionisation (ESI) negative and positive modes.

Peak	Solvent	RT (min)	Component name	Observed neutral mass (Da)	Detector counts	Adducts	Formula	MS/MS Fragments	ES mode	Ontology or class	References
1	Acetone	4.5	Agosterol A	576.3636	47 692	+H	$C_{33}H_{52}O_8$	-	+	Lipid	-
2	Acetone	5.2	Vernolepin	276.206	45 949		$C_{15}H_{16}O_5$	-	+	Sesquiterpene	Alara et al. (2017)
3	Acetone	5.8	Vernodalol	392.41	47 692	+H	$C_{20}H_{24}O_8$	-	+	Sesquiterpene	Erasto et al. (2006)
4	Acetone	5.9	Kaempferol-3-O-glucuronoside	462.4829		−H	$C_{21}H_{18}O_{12}$	285.0405, 150, 461.072, 229.0511, 230.0, 113.0247, 329.23	−	-	Yuan et al. (2008) Panizzi et al. (2002)
5	Acetone	5.99	Oleanolic acid	456.3642	45 672	−H, +Cl	$C_{3O}H_{48}O_3$	203.0, 250, 202.0, 249, 189.0, 260 320.0, 130, 190.0, 266	−	Lipid	Corey and Lee (1993)
6	Acetone	7.4	Apigenin	270.021	46 008	H, +Na	$C_{15}H_{10}O_5$	117.038, 150, 269.052, 151.008, 113 149.029, 119	+	Flavonoid	Alara et al. (2017)
7	Acetone	8.1	Thiamine	265.36	46 415	+H, +Na	$C_{12}H_{17}N_4OS+$	263.11, 2, 233.2, 0.28, 147.1, 115.2, 171.00, 90	+	Terpenoid	Alara et al. (2017)
8	Acetone	8.53	Arctiin	276.1728	282 33 0	-	$C_{17}H_{24}O_3$		+	Glycoside	Arslanyolu and Erdemgil (2006)
9	Acetone	9.1	Vernomenin acetate	318.41	45 189	+H, +Na	$C_{17}H_{18}O_6$	-	+	Sesquiterpene	Alara et al. (2017)
10	Acetone	11.2	Ascorbic acid	176.08	46 319	+H, +Na	$C_6H_8O_6$	176.0, 99.99, 72, 116.0, 59.2, 15.04, 159.0, 43.0, 130, 164.3, 10.5, 85.0, 91, 164.3, 9.73, 141.0, 33.5, 160.5, 9.46, 147.0, 28.3, 100, 75.2, 117.0, 55.3, 118.3, 48.85, 133.0, 32.5, 151.6, 22.62, 1.0, 174.5, 146.35, 17.32	+	Terpenoid	Alara et al. (2017)
11	Acetone	12.24	Cholic acid	408.60	52 183	+H	$C_{24}H_{40}O_5$	372.09, 20, 271.0, 129, 310.5, 312, 90, 318, 86.21, 150, 253.0, 64.75, 324.0 350.2, 55.0, 323.6	+	-	Horai et al. (2010)
12	Acetone	12.63	10-*O*-Methyl alismoxide	316.203	20 964	+H	$C_{20}H_{28}O_3$	80.0, 252.2, 149.1	+	Terpenoid	Jin et al. (2012)
13	Acetone	12.66	Aspersteroid A	436.2614	19 774	+H	$C_{28}H_{36}O_4$	220.0, 137.4, 135.7, 77.5, 70.1	+	Terpenoid	Jin et al. (2012)
14	Acetone	14.21	Oxymatrine	264.301	124 615	+HCOO	$C_{15}H_{21}N_2O_2$	148.1	−	Alkaloid	Liu et al. (2014)
15	Acetone	15.88	Notoginsenoside R2	770.4847	41 641	+HCOO	$C_{41}H_{70}O_{13}$	609.4347, 162, 639.4401, 130, 477.3912, 290, 457.7413, 309	−	-	Zhang et al. (2019)
16	Acetone	15.63	6,9-Octadecanedioic acid methyl ester	290.2233	90 957	+Cl	$C_{19}H_{34}O_2$	-	−	-	-
17	Acetone	16.01	Etiocholanolone	290.2246	54 341	+Cl	$C_{19}H_{30}O_2$	-	−	Steroid	-
1	Ethanol	4.13	Quercetin deoxyhexoside	253.370	15 419	-	$C_{21}H_{19}O_{11}$	253, 240.2, 10.9	+	Flavonoid	Tsamo, Ndibewu and Dakora (2018)
2	Ethanol	4.8	Quercetin	301.0352	16 238	+Na, +H	$C_{16}H_9O_7$	179, 151, 112.35	−	Flavonoid	Tsamo et al. (2018)
3	Ethanol	5.29	Mellein	178.0631	15 307	+Na, + H +NH4	$C_{10}H_{10}O_3$	161, 10.05, 178,133, 140.5, 171, 6.35	+	-	Reveglia, Masi and Evidente (2020)
4	Ethanol	7.97	Luteolin	286.0477	18 414	+H, Na	$C_{15}H_{10}O_6$	119.0, 1, 168.2, 200.0, 85.73, 161.0, 123.36, 258.0, 27.23, 262.0, 24.12	−	Flavonoid	Hasibuan et al. (2020)
5	Ethanol	7.97	2-Methoxycinnamic acid	178.0631	18 351	+NH4, + Na, +H	$C_{10}H_{10}O_3$	172, 5.99, 147, 30.77, 91, 88.73, 118, 59.92, 77, 100.83	−	-	Hasibuan et al. (2020)
6	Ethanol	8.2	Linoleic acid	277.2148	16 742	H, Na	$C_{18}H_{29}O_2$	-	+	Fatty acid	Tsamo et al. (2018)
7	Ethanol	12.23	Silenoside A	642.365	17 060	+H	$C_{18}H_{35}O_{12}$	-	−	Saponin	Mamarasulov, Davranov and Jabborova (2020)
8	Ethanol	13.19	Oleanolic acid	320.245	17 088	+Na, +H	$C_{20}H_{32}O_3$	92.9, 220, 40.7, 265.78, 172.1, 302.5, 47.5, 257.1	−	Fatty acid	Zhanzhaxina and Suleimen (2021)
9	Ethanol	13.41	Stearic acid	283.2622	16 773	+H	$C_{18}H_{35}O_2$	-	+	Fatty acid	Tsamo et al. (2018)
10	Ethanol	13.5	7α-hydroxy-8(17)-labden-15-oic acid (Salvic acid)	322.2499	16 409	+Na, +H, +K	$C_{20}H_{34}O_3$	-	+	Terpenoid	-
11	Ethanol	13.65	Funkioside C	738.417	18 271	+H	$C_{39}H_{62}O_{13}$	-	+	Saponin	-
12	Ethanol	14.64	Glycerol monostearate	358.3081	14 841	+Na, +H, +K, +NH4	$C_{21}H_{42}O_4$	147.01, 210.2, 100, 255.36, 117.34, 205.8, 267, 85.6	+	Fatty acid	Kind et al. (2009)



ESI, electrospray ionisation; ESI+, electrospray ionisation positive; ESI-, electrospray ionisation negative.

FIGURE 2: (a) is a chromatogram of *V. amygdalina* Delile in acetone (positive mode); (b) is a chromatogram of *V. amygdalina* Delile in acetone (negative mode); (c) is a chromatogram of *V. amygdalina* Delile in ethanol (positive mode); (d) is a chromatogram of *V. amygdalina* Delile in ethanol (negative mode).

fatty acids, might also contribute to the reported antifungal activity in our study. Similarly, Ivanescu, Miron and Corciova (2015) explained that the mechanism of biological

TABLE 2: A summary of bioactive chemical classes identified in acetone and ethanoic extracts of *V. amygdalina* Delile.

Plant name	Class	Acetone	Percentage	Ethanol	Percentage
V. amygdalina	Terpenoids	4	23.53	1	8.33
	Fatty acids	-	-	4	33.33
	Alkaloids	1	5.88	-	-
	Flavonoids	1	5.88	3	25
	Sesquiterpenes	3	17.65	-	-
	Saponins	-	-	2	16.67
	Lipids	2	11.76	-	-
	Glycosides	1	5.88	-	-
	Steroids	1	5.88	-	-
	Other	4	23.53	2	16.67
	Total	**17**	**100**	**12**	**100**

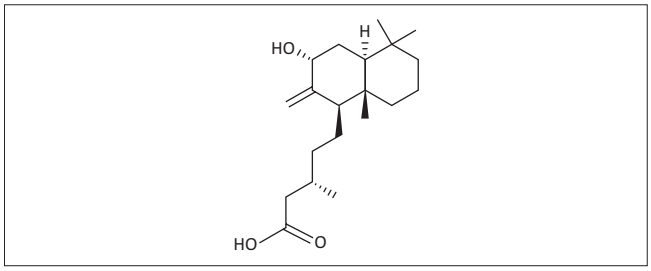


FIGURE 3: Salvic acid chemical structure.

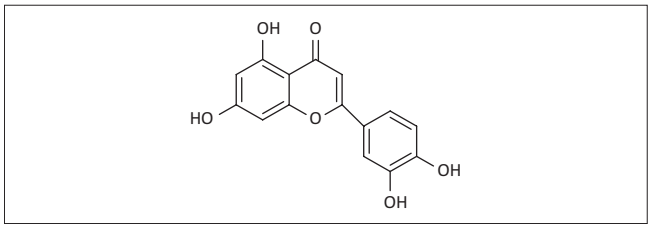


FIGURE 4: Luteolin chemical structure.

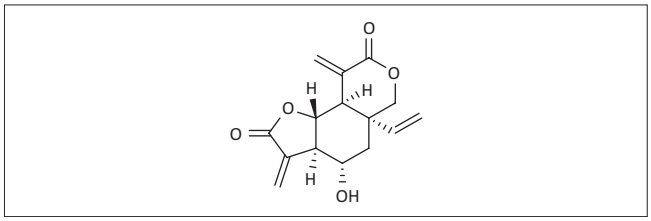


FIGURE 5: Vernolepin chemical structure.

activity of alkylating sesquiterpene lactones and the nucleophile sulfhydryl group in proteins led to the disruption of cell function that caused cell wall damage of the fungus. Sesquiterpenes are highly identified and documented in the *Vernonia* genus (Oladele et al. 2021). This study discovered sesquiterpenes such as vernolepin (Figure 5), vernodalol and vernomenin acetate (peaks 2, 3, and 9), with mass ions of 276.206, 392.41 and 318.41 m/z, at 5.2 min, 5.8 min and 9.1 min, respectively, from acetone leaf extract of *V. amygdalina*, which are widely used in traditional medicine to treat fungal infections. Peak 8 was identified as vernodalol, with an ion mass of 392.41 m/z, at 5.8 min from the acetone leaf extract, and it was found to have potent antifungal activity against *Penicillium*

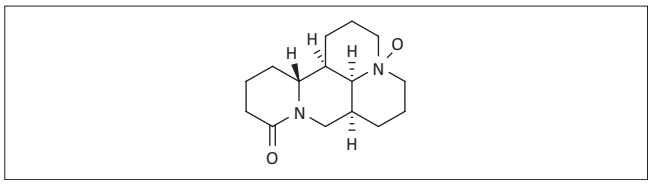


FIGURE 6: Oxymatrine chemical structure.

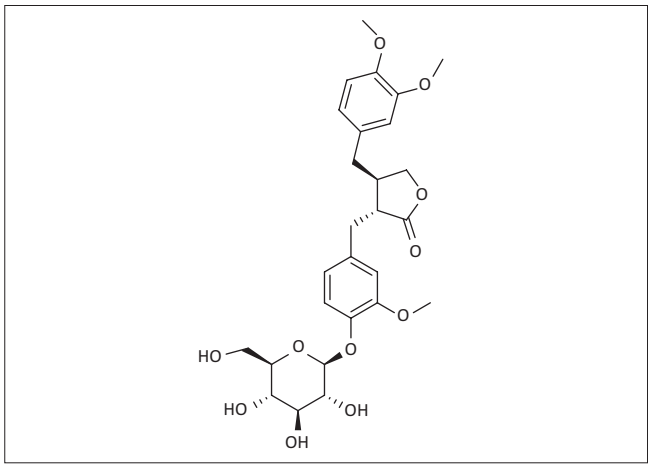


FIGURE 7: Arctiin chemical structure.

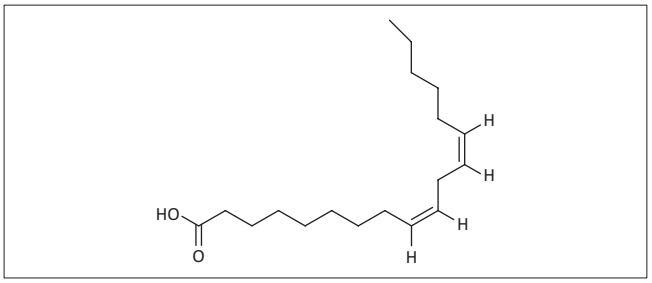


FIGURE 8: Linoleic acid chemical structure.

notatum and *Aspergillus flavus* (Erasto, Grierson & Afolayan 2006).

Alkaloids

Alkaloids have been shown to have potential value as antimicrobial agents for plant protection (Compean and Ynalvez 2014). They reportedly have the strongest pharmacological effects (Bribi 2018). Several studies report this group of compounds as the most active group with considerable biological activities, including toxicity to other organisms (Jain, Khatana & Vijayvergia 2019). Despite reports of the group's toxicity, which limits its use in clinical investigations, it remains an important group as active plant defenders against plant diseases (Jain, Mohammadi & Wallace 2019). Peak 14 from the acetone leaf extract of the *V. amygdalina* plant species was tentatively identified as oxymatrine (Figure 6), with the mass of the uncharged parent 264.301 m/z at 12.21 min. This compound has been reported to possess *in vitro* antifungal properties against *Fusarium oxysporum*, *Valsa pini* and *Cladosporium oxysporum*. Yang and Zhao (2006) discovered that oxymatrine had a substantial inhibitory impact against *Fusarium oxysporum*, with EC50 values of 26 g mL^{-1}.

Glycosides

Peak 8 in acetone leaf extracts of *V. amygdalina* Delile was tentatively identified as arctiin, having ion mass 276.1729 m/z at 8.53 min (Table 1, Figure 7). This compound has been explored against bacterial infections but not against fungal pathogens; hence, it is necessary to isolate this compound and investigate its antifungal effects. Previously, an *in vitro* test utilising an ethanol crude extract of *V. amygdalina* at 300 mg/mL significantly prevented the growth of *Cercosporella persica* and *Curvularia lunata* (Ilondu 2013). Like terpenes, plant glycosides show a remarkable degree of sensitivity against a wide variety of fungal species, and the effects are not restricted to one plant family or class of glycosides (Khan et al. 2017). Favel et al. (2005) reported that the antifungal mechanism of glycosides is attributable to their capacity to form complexes with the sterols of fungal membranes, therefore producing spore-like structures that tear the membrane, resulting in the death of fungal cells. Although glycosides can destroy fungal spores, their antifungal properties are not as well known.

Fatty acids

Peaks 6, 8, 9 and 12 were tentatively identified as linoleic acid, oleanolic acid (Figure 8), stearic acid and glycerol monostearate in ethanol leaf extract of *V. amygdalina* Delile, giving ion mass 277.2148, 320.245, 283.2622 and 358.3081 m/z at 8.2 min, 13.19 min, 13.41 min and 14.64 min, respectively (Table 1). These compounds are characterised as fatty acid and have been reported to possess increased fungicidal activity against *Rhizoctonia solani*, *Pythium ultimum* and *Pyrenophora avenae* due to their increased freedom of movement inside the fungal membrane (Pohl, Kock, Thibane 2011; Walters et al. 2004). There were no fatty acid components found in the acetone leaf extract. For decades, fatty acids have received widespread recognition for their antimicrobial properties (Fan et al. 2012). The majority of studies on fatty acids have involved their innate immunity function on humans and animals to protect against microbial pathogens (Kanai & Kondo 1979; Thormar & Hilmarsson 2007). Fatty acids are known to protect plants from photogenic colonisers, just as they do for humans and animals, and they may also play a part in protecting plants from microbial plant infections (Desbois & Smith 2010; Zasloff 2011) In addition to their potent inhibitory activities against fungal crop pathogens, fatty acids as antifungal agents are noncorrosive compared to other antifungal substances and disinfectants (Walters et al. 2004; Whittle & Basketter 1993) that may otherwise alter the health of crops and cause toxicity to humans and the environment. The findings of Liu et al. (2008) suggest that fatty acids should be investigated as potential compounds efficient against fungal infections in agricultural crops.

Conclusion

The findings of this investigation revealed that *Vernonia amygdalina* Delile acetone leaf extracts contain more bioactive components than ethanol leaf extracts. In this work, the HPLC–QTOF/MS approach was used to detect and characterise active components, and it proved to be a practical method for identifying fragmentation pathways of

metabolites in leaf extracts, as well as being more sensitive than the previously reported methods. *Vernonia amygdalina* Delile is indicated for antifungal activity because it contains metabolites responsible for antifungal activity; nevertheless, more *in vitro* studies are needed to validate its efficiency.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

All the authors designed the study, coordinated data collection, carried out all the field work and drafted the manuscript.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability

The data used to support the findings of this study may be released upon application to the corresponding author, W.O-M.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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