Pak. J. Bot., 56(3): 979-988, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-3(2)

SECONDARY METABOLITE PROFILING IN *LEONOTIS NEPETIFOLIA* LEAF ACETONE AND ETHANOL EXTRACTS USING UPLC-qTOF-MS

VUSANI MAKANANISE[1], CHRISTELDAH MKHONTO[1], IDOWU JONAS SAGBO[1,2], MADONNA NGWATSHIPANE MASHABELA[1,5*], PETER TSHEPISO NDHLOVU[1], BONGANI PETROS KUBHEKA[3,4] AND WILFRED OTANG-MBENG[1]

[1]*School of Biology and Environmental Sciences, University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa*
[2]*Department of Chemistry, Cape Peninsula University of Technology, Bellville Campus, Bellville, South Africa*
[3]*Plant & Crop Production Research Directorate, Döhne Agricultural Development Institute, Stutterheim, South Africa*
[4]*University of KwaZulu Natal, Plant Pathology Department, Private Bag X01, Scottvill, 3209, South Africa*
[5]*Department of Soil Sciences, Soil Cultivation, Agricultural Research Council Infruitec-Nietvoorbij, Stellenbosch, South Africa*
[*]*Corresponding author's email: ngwatsipanemashabela@gmail.com*

Abstract

Natural compounds derived from medicinal plants, as well as their plant extracts, are utilized to treat a variety of ailments in both human and plant pathology. Using the ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-qTOF-MS), data were analyzed in both negative and positive ionization modes. A total of 19 metabolites between 7.62 and 17 minutes in acetone extracts. While 20 metabolites between 6.69 and 11.07 minutes in ethanol extracts of *Leonotis nepetifolia* were tentatively identified according to their retention times and fragment ions. Hirsutine and tuberostemonone were some of the identified secondary metabolites in acetone extracts and have previously been evaluated for their antifungal activity against phytopathogenic fungi. Flavonoids were the most prevalent secondary metabolites in the current study with 26.3% and 22.2% in ethanol and acetone extracts respectively. Based on the effectiveness of the solvent used, the summary of the chemical classes indicates that ethanol is better than acetone extract. Based on the major findings detected in this study, the complex profile of secondary metabolites in *Leonotis nepetifolia* opens new horizons to the industrial use of this plant species, which could represent therapeutic potential and antifungal activities. Our findings suggest that the presence of phytochemicals can be linked to their medicinal properties.

Key words: Medicinal Plants; Phytochemicals; Secondary metabolites; Antifungal activities.

Introduction

Medicinal plants are plants that possess chemicals that can be utilized for therapeutic purposes or are precursors of chemo-pharmacological semi-synthetic novel drugs (Sharanabasappa *et al*., 2007). These plants have been used as a source of medicine in all societies since time immemorial (Mahomoodally *et al*., 2005). In Kenya different plant parts have been employed as antifungal, antioxidant, hormonal, and enzyme treatments for various illnesses (Karinge, 2006). Secondary metabolites are tiny organic compounds formed during plant growth from primary metabolites (Altemimi *et al*., 2017). These compounds are considered fascinating due to their structural diversity and potency as therapeutic candidates (Twaij & Hasan, 2022). These compounds include alkaloids, glycosides, amines, insecticides, steroids, flavonoids, and related metabolites, which are abundant in medicinal plants and have been widely exploited in the drug and pharmaceutical industries (Saxena *et al*., 2013). Pharmaceutical industries continuously discover the therapeutic potential of medicinal plants due to the high amount of secondary metabolites.

The use of medicinal plants in agriculture to control specific diseases is becoming more common due to their high content of secondary metabolites (Martinez, 2012). Disease management with medicinal plants is becoming increasingly popular in farming (Meissle *et al*., 2010). As a result, most farmers in developing nations have turned to medicinal plants since they are less expensive and more readily available than synthetic fungicides, which are costly and have negative environmental consequences.

It has been shown in the literature that medicinal plants can be used as bio-fungicides (Bander, 2011). Medicinal plants are beneficial against fungal diseases in crops such as *Pythium* and are being researched further as a potential solution for root rot management (Baraka *et al*., 2011). *In vitro*, aqueous extracts of *Zygophyllum fabago* and ethanolic extracts of *Allium sativum*, *Azadirachta indica*, and *Curcuma longa* inhibit *Pythium aphanidermatum* mycelial growth (Parveen & Sharma, 2015). *In-vivo* testing of Zimmu leaf extract against *Pythium* revealed that it was active (More *et al*., 2017). Extracts of *Thymus vulgaris* and *Zingiber officinale (ginger)* are used to treat tomato damping-off diseases and may be used as an alternative natural product to control *Pythium* and *Fusarium* species and avoid the use of chemical fungicides (Balakrishnan *et al*., 2003). An aqueous extract of *Usnea pictoides* was found to inhibit *Pythium aphanidermatum* found in the rotten ginger rhizome (Chapagain *et al*., 2007). Plants contain phytochemicals that help in the prevention of diseases and the promotion of plant health. Phytochemicals are active compounds that have therapeutic characteristics and are therefore regarded as medicines or drugs (Shakya, 2016).

Leonotis Nepetifolia is known as the "Christmas candlestick" in Eastern Cape, South Africa and widely dispersed throughout Southern Africa (Tonisi *et al*., 2020). This herb's stems emerge from a strong wood foundation. The green leaves on the stems grow opposite each other and feature a lot of glandular trichomes on the leaf lamina (Mazimba, 2015). The herb has clusters of orange, apricot, or white blooms that resemble the lion's ears, hence the name "nepetifolia (lion coloured)". Birds, bees, and butterflies are attracted to the nectar produced

by the flowers (Mazimba, 2015). Native South Africans in the Eastern Cape Province have benefited greatly from *L. nepetifolia* (Mazimba, 2015). Treatment of snakebites, headaches, wounds, bronchitis, high blood pressure, chest pains, epilepsy, influenza, and menstrual cycle period aches are among the traditional applications of *L. nepetifolia* (Rattray & Van Wyk, 2021). *L. nepetifolia* is a valuable commercial plant. In the current study, the medicinal plant was chosen based on its reported antifungal properties based on a review of existing literature and evaluation of the activities of crude plant extracts using invitro analysis (Dhawan *et al*., 2013).

Oliveira *et al*., (2015) investigated the potential cytotoxicity and antibacterial mode of action of the plant's hydroethanolic extract from the leaves, as well as phytochemical analysis. Tidke *et al*., (2021) conducted a review of *L. nepetifolia*'s chemical composition, pharmacological activity, and medical significance. Sobolewska *et al.,* (2012) identified terpene compounds, flavonoids, tannins, iridoids, sterols, and fats in methanolic and acetone extracts of *L. nepetifolia.* The extracts had no effect on specific strains of Gram (+) and Gram (-) bacteria, though. Gram positive and Gram negative bacteria were used as test subjects for the antibacterial activity of the essential oil of *L. nepetaefolia,* and it was discovered to be moderately active. The antimicrobial potential of *L. nepetifolia* remains underexplored hence few studies were reported (Kamatou *et al*., 2006). Because the existence of these bioactive chemicals confers medicinal qualities on this plant, many of them have been extensively examined and evaluated for antimicrobial, antioxidant, antibacterial, cathartic, and anti-cancer action, among others (Shakya, 2016). As a result, the current study aims to profile bioactive secondary metabolites in *L .nepetifolia* acetone and ethanol leaf extracts using the UPLC-qTOF-MS.

Materials and Methods

Plant collection, identification and extraction: The leaves of *Leonotis nepetifolia* plant species were collected in September 2020 along the roadside of University of Mpumalanga, Nelspruit, South Africa (25.4371° S, 30.9818° E). Plant specimens were identified, and voucher specimen (VM001) was prepared and deposited at the South African National Biodiversity Institute (SANBI) herbarium in Pretoria for authentication. *L. nepetifolia* leaves were separated from the rest of the plant and cleaned with clean tap water to eliminate unwanted materials. The leaves were then rinsed with distilled water and oven dried for 72 hours at 40°C to a consistent weight. The leaves were further pulverized to a homogeneous powder using a sterile electric blender (Commercial Blender type GB27, Hamilton Beach Brands, Inc. China). The powdered samples were then stored in airtight containers to preserve the biomolecules present in the plant and stored at room temperature for 4 days. 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 on an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 h. The extracts were thereafter filtered using a Buchner funnel and Whatman No. 1 filter paper, and the filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH and Co, Germany) at 40°C (Otang *et al*., 2012). Each extract was exposed to fan air for solidification (*Saleh-e-In* and *Staden*, 2018).

UPLC-qTOF-MS profiling: A 0.22-µm polytetrafluorethylene filter was used to filter the supernatants. A Quadrupole 120 time-of-flight (QTOF) mass spectrometer UPLC-qTOF/MS (Waters, Milford, MA, USA) was used to identify and quantify predominant secondary metabolites. An ACQUITY UPLC BEH C18 column (2.1 × 100 mm i.d., 1.7×10^{-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; 25–30 min, 95% A. The flow rate of the mobile phase was 0.4 mL/min and the temperatures of the column and autosampler were maintained at 30 and 10°C, respectively. Data were analysed in both negative and positive ionization modes. Data were processed using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) (Lai *et al*., 2018). Functions 1 (unfragmented channel) and 2 (fragmented channel) of the Waters MSe data were processed by MSDial 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 the majority of these compounds, the peak height intensity was converted to concentration in a semi-quantitative manner by interpolation of a calibration curve for catechin acquired under the same instrumental conditions. Each deconvoluted feature (alignment in MSDial), together with its associated MS1 and MS spectra was exported from MSDial to MSFinder. 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 the Markerlynx v4.1, alignment and peak detection and raw data filtering were conducted. A mass range of 100–1000 Da, 5–21 min retention time as well as 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

Quantification of non-targeted metabolites in *Leonotis nepetifolia*: The predominant secondary metabolites in *L. nepetifolia* are shown in Tables 1 and 2. Secondary metabolites of *L. nepetifolia* were identified using UPLC-qTOF-MS. Data were analyzed in negative and positive ionization modes (Figs. 1 and 2). A total of 19 bioactive metabolites were detected between 2.99 and 15.84 min in the acetone leaf extracts of *L. nepetifolia*. While a total of 20 bioactive metabolites were detected between 5.8 and 15.15 min in ethanol leaf extracts of *L. nepetifolia*. Compounds were tentatively identified according to their molecular formula, retention time and their fragment ions in comparison with data from the literature. Flavonoids (22.2%), Alkaloids (16.7%), and Terpenes (16.7%) were the most abundant phytoconstituents in the acetone leaf extract; whereas Flavonoids (26.3%) and Glycocydes (15.8%) were the most abundant in the ethanol leaf extract (Table 3). The presence of these phytochemicals in plants confers distinct therapeutic qualities. As a result, the presence of the aforementioned phytochemicals in *L. nepatifolia* can be linked to their therapeutic potential and the effectiveness of the solvent used (Imran *et al*., 2012). In this study, ethanol extract was the best with more chemical classes identified (between 5. 6 and 22.2%) than acetone extract (Table 3). Due to its highly polar nature, ethanol extract had more secondary metabolites identified in it while ethanol had fewer secondary metabolites identified. This confirmed conclusions reached earlier by Borges *et al*., (2020).

Alkaloids: In contrast to the ethanol extract, which contained no alkaloids, the acetone leaf extract of *Leonotis nepetifolia* included the alkaloids hirsutine, tuberostemonone, and agroclavine. Alkaloids are essential for plant protection and survival since they defend plants from other plants as well as microorganisms (through antibacterial and antifungal activities), insects, and herbivores (via feeding deterrents), as well as assure their survival (Saxena *et al*., 2013). Therefore, the presence of alkaloids in the plant species studied in the current investigation ensures their antimicrobial ability. Peak 6 in acetone leaf extracts was characterized as hirsutine, and had a molecular formula $C_{22}H_{28}N_2O_3$ displaying the mass of the uncharged parent compound (m/z) 368.50 at 8.28 min (Fig. 1). Hirsutine is a natural alkaloid. Peak 7 was characterized as tuberostemonone with a molecular formula $C_{22}H_{31}NO_6$ and the mass of the uncharged parent compound (m/z) 405.50 at 9.38 min. Tuberostemonone is a limonoid present in the seeds of most plants from the stemonaceae family. It possesses antifungal properties that make it a biological therapeutic agent. Tuberostemonone, isolated from *Stemona sessilifolia* has been tested against *P. palustris.* This natural compound has also been evaluated for its antifungal activity against 6 phytopathogenic fungi: *Rhizoctonia solani*, *Erysiphe graminis*, *Pyricularia grisea*, *Botrytis cinerea*, *Puccinia recondite* and *Phytophthora infestans* (Liu *et al*., 2021). The results from the previous studies support that tuberostemonone from the crude extract of *Leonotis nepetifolia* plant species could be adopted for biological control and management of plant diseases. Agroclavine is a natural alkaloid characterized by peak 10 displayed m/z 238.30 at 13.58 min with chemical formula $C_{16}H_{18}N_2$ and the product ion (fractions) range are 136.43, 158.37, 165.57, 179.97, 201.57.

Flavonoids: Flavonoids antioxidant, anti-inflammatory, and antibacterial properties have been thoroughly documented in literature. Flavonoids have recently received a lot of attention due to their various biological and pharmacological activities. Flavonoids possess antibacterial, cytotoxic, anti-inflammatory, and anticancer activity (Tapas *et al*., 2008). The current investigation found that flavonoids were the most abundant class of compounds in *Leonotis nepetifolia* leaf extracts. In the current study Shuncilin, Catechin-(4α→8)-catechin, Kaempferol-3-glucuronide and Quercetin-5-O-β-D-glucopyranoside [Peak 3, 11, 17and 18 respectively] were tentatively identified in acetone leaf extract and compound Kaempferol-4-glucuronide, Quercetin-5-O-β-D-glucopyranoside, Iridin, Viscumneoside VI, Quercetin-4′-glucoside and 5-Ene-methylcholate-3-O-β-D- glucopyranoside [3, 4, 8, 9, 11 and 15 respectively] were tentatively identified in the ethanol leaf extract.

Peak 15 characterized as 5-Ene-methylcholate-3-O-β-D-glucopyranoside is a natural flavonoid with chemical formula $C_{31}H_{50}O_{10}$ displaced as 582.70 at 15.07 min (Fig. 2). It was one of five flavonoids extracted from the leaves of *Mangifera indica*, and its antifungal activity was evaluated against five fungus species namely *Alternaria alternata Keissler*, *Aspergillus fumigatus Fresenius*, *Aspergillus niger van Tieghem*, *Macrophomina phaseolina (Tassi) Goid* and *Penicillium citrii*. The flavonoids were found to considerably reduce fungal growth in the study Kanwal *et al*., (2010). We, therefore, conclude based on our results that *Leonotis nepetifolia* may inhibit the growth of the pathogen and may be used for antifungal activity due to the presence of flavonoids.

Terpenes: Terpenoids are the most structurally varied group of plant secondary metabolites; they operate as phytoalexins indirect plant defence or as signals in indirect defence responses involving herbivores and other natural enemies (Vaughan *et al*., 2018). Peak 5 in acetone leaf extracts was characterized as Oriediterpenol had a molecular formula $C_{20}H_{32}O_2$ displaying the mass of the uncharged parent compound (m/z) 304.50 at 7.7 min (Fig. 1), while Peak 5 in ethanol leaf extract was characterized as achillin, with molecular formula $C_{15}H_{18}O_3$ displaying the mass of the uncharged parent 246.30 at 7.63 (Fig. 2). Terpenes offer therapeutic effects such as anticancer, antimalarial, anti-ulcer, hepaticidal, antibacterial, diuretic, and anticarcinogenic (Tholl *et al*., 2004).

Glycosides: Peak 1 in acetone leaf extracts of *Leonotis nepetifolia* was tentatively identified as Khellol-β-D-glucoside, having an ion mass of 40.40 m/z at 2.99 min (Fig. 1), while the leaf extract of ethanol identified Cistanoside C (peak 2) (Fig. 2), 2, 4, 6-Trihydroxyacetophenone-2, 4-di-O-β-D-glucopyranoside (peak 10) and 6-Hydroxykaempferol-3-O-glucoside (peak 12). A study done by Anggraeni *et al*., (2022) confirmed that Khellol-β-D-glucoside may have antifungal activities.

Table 1. Tentative Identification of secondary metabolites present in acetone leaf extract of *Leonotis nepetifolia*.

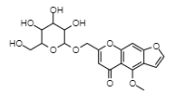
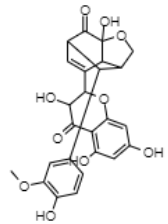
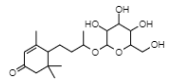
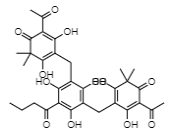
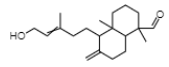
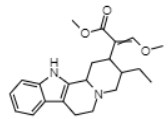
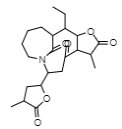
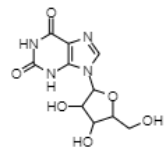
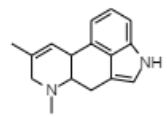
Peak No.	Identified compounds	Ontology	Retention time(min)	Error (ppm)	Chemical formula	Chemical structure	Molecular weight	ES mode	Adducts	Product ion m/z
1.	Khellol-β-D-glucoside	Glycoside	2.99	0,2	$C_{19}H_{20}O_{10}$		408,40	+	+H, +K, +Na	142.50, 149.70, 164.10, 166.44, 171.30, 174.98, 387.98, 396.56, 400.45 404.34
2.	Silydianin	Tyrosine	3.5	2.6	$C_{25}H_{22}O_{10}$		482,40	+	+HCOO	91.69 210.26 216.18 224.72 246.31 382.25 388.12 395.89 492.42
3.	shuncilin	Flavonoid	3.9	-0.9	$C_{19}H_{32}O_7$		372,50	+	+H	92.75, 123.16, 136.69, 147.72, 245.25
4.	Filixic acid ABA	Fatty acid	7.5	-8.4	$C_{32}H_{36}O_{12}$		612,60	-	+HCOO	107.49, 205.24, 230.51, 358.62, 358.77371.24, 383.71, 396.18 516.05
5.	Oriediterpenol	Terpene	7.7	-6.8	$C_{20}H_{32}O_2$		304,50	-		_
6.	Hirsutine	Alkaloid	8.2	7.5	$C_{22}H_{28}N_2O_3$		368.50	+	+Cl	125.47, 175.83, 178.82, 192.52, 195.51, 212.53
7.	Tuberostemonone	Alkaloid	9.4	-1.2	$C_{22}H_{31}NO_6$		405.50	+	+H	109.25, 133.46, 146.62, 159.43, 189.4, 203.56, 228.75, 383.32, 394.58, 396.60, 399.59 400.44, 401.15
8.	Xanthosine	Nucleoside	11.11	3.6	$C_{10}H_{12}N_4O_6$		238.30	+	$+CH_3COO$	119.16 136.31 141.18 144.65 152.83 218.84
9.	Stearidonic acid	Lineolic acid	12.23	0.3	$C_{18}H_{28}O_2$		276.40	-	+H	153.93 173.20 180.40 184.07
10.	Agroclavine	Alkaloid	13.58	7.0	$C_{16}H_{18}N_2$		238.30	-	$+CH_3COO$	136.43, 158.37, 165.57, 179.97, 201.57

Table 1. (Cont'd.).

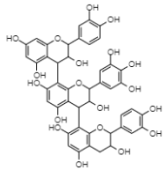
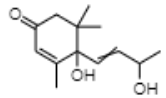
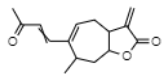
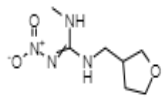
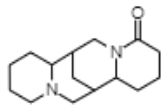
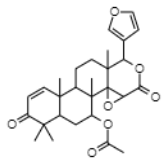
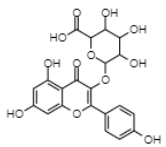
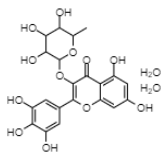
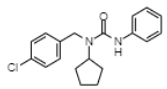
Peak No.	Identified compounds	Ontology	Retention time(min)	Error (ppm)	Chemical formula	Chemical structure	Molecular weight	ES mode	Adducts	Product ion m/z
11.	Catechin-(4α→8)-catechin	Flavanol	13.67	-1.9	$C_{30}H_{26}O_{12}$		578.50	+	-H	_
12.	Vomifoliol	Oxidoreductase	13.88	6.6	$C_{13}H_{20}O_3$		224.30	+	+Na	142.85, 143.40, 148.27, 151.95, 155.47, 169.87, 177.07, 191.47 195.15
13.	Xanthatin	Sesquiterpene	13.90	-1.2	$C_{15}H_{18}O_3$		246.30	+	+H, +Na, +NH_4	56.43, 143.87, 153.53, 162.51, 176.49, 181.36, 182.68, 185.89, 194.13
14.	Dinotefuron	Neonicotinoid	14.00	-6.8	$C_7H_{14}N_4O_3$		202.21	+	+Cl	145.64, 145.90, 153.10, 192.36,
15.	Lupanine	Quinolizidine	14.05	-8.9	$C_{15}H_{24}N_2O$		248.36	-	+HCOO	141.40, 196.60, 175.27, 189.31, 184.29, 192.83
16.	Gedunin	Triterpenoid	14.66	3.6	$C_{28}H_{34}O_7$		482.60	-	+Na	77.09, 110.45, 154.21, 159.08, 162.75, 223.88, 227.55, 377.33 468.51, 480.98 , 484.87
17.	Kaempferol-3-glucuronide	Flavonoid	15.61	1.8	$C_{21}H_{20}O_{12}$		464.40	+	+H, + K, +Na	110.73, 153.93, 62.47 173.20, 194.80, 216.40227.27, 370.10, 427.21431.10, 434.99, 443.57, 456.04
18.	Quercetin-5-O-β-D-glucopyranoside	Flavonoid	15.78	8.1	$C_{21}H_{20}O_{12}$		464.30	+	-H	_
19.	Pencycuron	Monochlorobenzenes	15.84	-8.7	$C_{19}H_{21}ClN_2O$		328,80	-	+H, +K, +Na	136, 160.27, 168.82, 179.54, 202

Table 2. Tentative Identification of secondary metabolites present ethanol leaf extract of *Leonotis nepetifolia*.

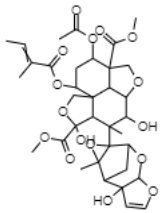
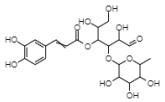
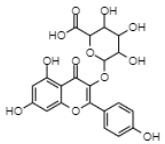
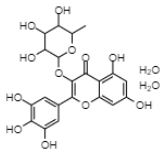
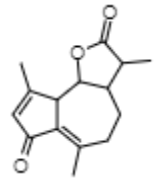
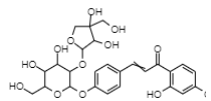
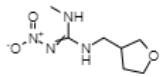
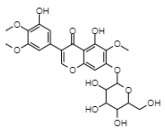
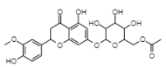
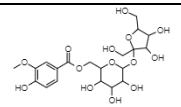
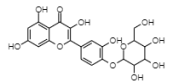
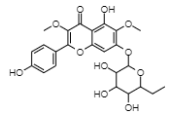
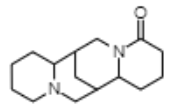
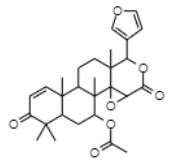
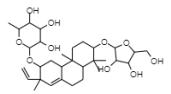
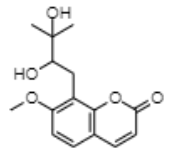
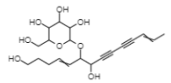
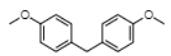
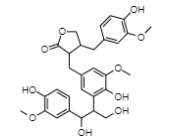
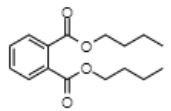
Peak No.	Identified compounds	Ontology	Retention time(min)	Error (ppm)	Chemical formula	Chemical structure	Molecular weight	ES mode	Adducts	Product ion m/z
1.	Azadirachtin	Limonoid	5.8	-3.8	$C_{35}H_{44}O_{16}$		720.70	+	+Cl	92.75, 123.16, 136.69, 139.19, 203.03, 211.57, 245.25, 380.34, 394.57, 399.50, 413.68, 477.99, 485.77, 494.33
2.	Cistanoside C	Glycoside	6.01	-7.8	$C_{30}H_{38}O_{15}$		638.60	+	-H, +Cl	_
3.	Kaempferol-4-glucuronide	Flavonoid	6.02	1.8	$C_{21}H_{20}O_{12}$		464.40	+	+H, +K, +Na	110.73, 153.93, 62.47 173.20, 194.80, 216.40227.27, 370.10, 427.21431.10, 434.99, 443.57, 456.04
4.	Quercetin-5-O-β-D-glucopyranoside	Flavonoid	6.4	8.1	$C_{21}H_{20}O_{12}$		464.30	+	-H	_
5.	Achillin	Terpene	7.63	1.1	$C_{15}H_{18}O_3$		246.30	-	+H	126.31, 211.69
6.	Licurazide	Triterpene	7.68	-0.3	$C_{26}H_{30}O_{13}$		550.50	+	-H, +Cl	124.89, 144.08, 157.77, 161.37, 169.91, 213.11, 332.69, 398.96, 413.36, 417.81, 452.15, 459.93, 541.97
7.	Dinotefuron	Neonicotinoid	8.42	-6.8	$C_7H_{14}N_4O_3$		202.21	+	+Cl	145.64, 145.90, 153.10, 192.36,
8.	Iridin	Flavanoid	4.48	5.0	$C_{24}H_{26}O_{13}$		522.50	+	+Na	_
9.	Viscumneoside VI	Glycosyloxy flavone	5.16	5.9	$C_{24}H_{26}O_{12}$		506.50	+	+Na	132.33, 168.33, 176.87, 189.93, 198.47, 514.50, 205.67, 331.18, 522.28, 526.97, 534.75, 543.48

Table 2. (Cont'd.).

Peak No.	Identified compounds	Ontology	Retention time(min)	Error (ppm)	Chemical formula	Chemical structure	Molecular weight	ES mode	Adducts	Product ion m/z
10.	2,4, 6-Trihydroxyacetophenone-2, 4-di-O-β-D-glucopyranoside	Phenolic glycoside	5.16	7.5	$C_{20}H_{28}O_{14}$		492.43	+	+Cl	_
11.	Quercetin-4′-glucoside	Flavonoid	6.02	7.6	$C_{21}H_{20}O_{12}$		464.40	-	-H	87.452, 165.89, 174.44, 185.16, 192.36, 399.93, 409.06, 415.73
12.	6-Hydroxykaempferol-3-O-glucoside	Glycoside	6.14	7.5	$C_{21}H_{20}O_{12}$		464.40	-	-H	_
13.	Lupanine	Quinolizidine	10.57	-8.9	$C_{15}H_{24}N_2O$		248.36	-	+HCOO	141.40, 196.60, 175.27, 189.31, 184.29, 192.83
14.	Gedunin	Triterpenoid	11.52	3.6	$C_{28}H_{34}O_7$		482.60	-	+Na	77.09, 110.45, 154.21, 159.08, 162.75, 223.88, 227.55, 377.33 468.51, 480.98, 484.87
15.	5-Ene-methylcholate-3-O-β-D-glucopyranoside	Flavonoid	11.34	4.9	$C_{31}H_{50}O_{10}$		582.70	-	+K	_
16.	Meranzin hydrate	Benzopyran	12.34	-0.6	$C_{15}H_{18}O_5$		278.30	+	+H, +Na, +NH_4	_
17.	Lobetyolin	Polyyne	12.55	8.2	$C_{20}H_{28}O_8$		396.40	+	+CH_3COO	125.13, 158.80, 173.20, 180.40, 187.60, 182.73, 187.60, 191.27, 194.80, 212.87, 344.41, 359.41
18.	Dehydrolindestrenolide	Androstanoid	14.78	8.34	$C_{15}H_{16}O_2$		228.29	+	+H	_
19.	Isolappaol C	Sesquilignan	15.00	1.2	$C_{30}H_{34}O_{10}$		554.60	-	-H	102.76, 161.90, 166.77, 170.24, 173.97, 235.24, 370.36, 466.38, 470.27, 474.16, 482.74, 504.31, 554.60
20.	Dibutyl phthalate	Terpene	15.15	2.6	$C_{16}H_{22}O_4$		278.30	-	+Na, +H, +K	135.93, 171.93, 180.47193.53, 202.07

Sesquiterpenes: Peak 13 was tentatively identified as Xanthatin in acetone leaf extract, which displayed m/z 246.30 at 13.90 min with the chemical formula $C_{15}H_{18}O_3$ (Fig. 1). The ethanol leaf extract of *Leonotis nepetifolia* contained no sesquiterpenes metabolites, per the findings of the present investigation. Xanthatin and its analogues showed a wide range of outstanding biological properties, including antifungal, anti-inflammatory, and antimicrobial action (Zhi *et al*., 2022). Furthermore, xanthatin modified at the exo-methylene group demonstrated considerable antifungal action in prior research. These findings demonstrated xanthatin's enormous potential as a lead compound for the creation of botanically fungicidal compounds (Vaughan *et al*., 2018).

Table 3. Summary of chemical classes identified in acetone and ethanol extracts of *Leonotis nepetifolia*.

Plant name	Class	Acetone	Percentage	Ethanol	Percentage
Leonotis nepetifolia	Alkaloids	3.0	16.7	0.0	0.0
	Flavonoids	4.0	22.2	5.0	26.3
	Terpenes	3.0	16.7	3.0	15.8
	Fatty acids	2.0	11.1	0.0	0.0
	Glycosides	1.0	5.6	3.0	15.8
	Limonoid	0.0	0.0	1.0	5.3
	Polyyne	0.0	0.0	1.0	5.3
	Androstanoid	0.0	0.0	1.0	5.3
	Sesquilignan	0.0	0.0	1.0	5.3
	Benzopyran	0.0	0.0	1.0	5.3
	Neonicotinoid	1.0	5.6	1.0	5.3
	Quinolizidine	1.0	5.6	1.0	5.3
	Glycosyloxy flavone	0.0	0.0	1.0	5.3
	Monochlorobenzenes	1.0	5.6	0.0	0.0
	Oxidoreductase	1.0	5.6	0.0	0.0
	Tyrosine	1.0	5.6	0.0	0.0
	Total	**18.0**	**100.0**	**19.0**	**100.0**

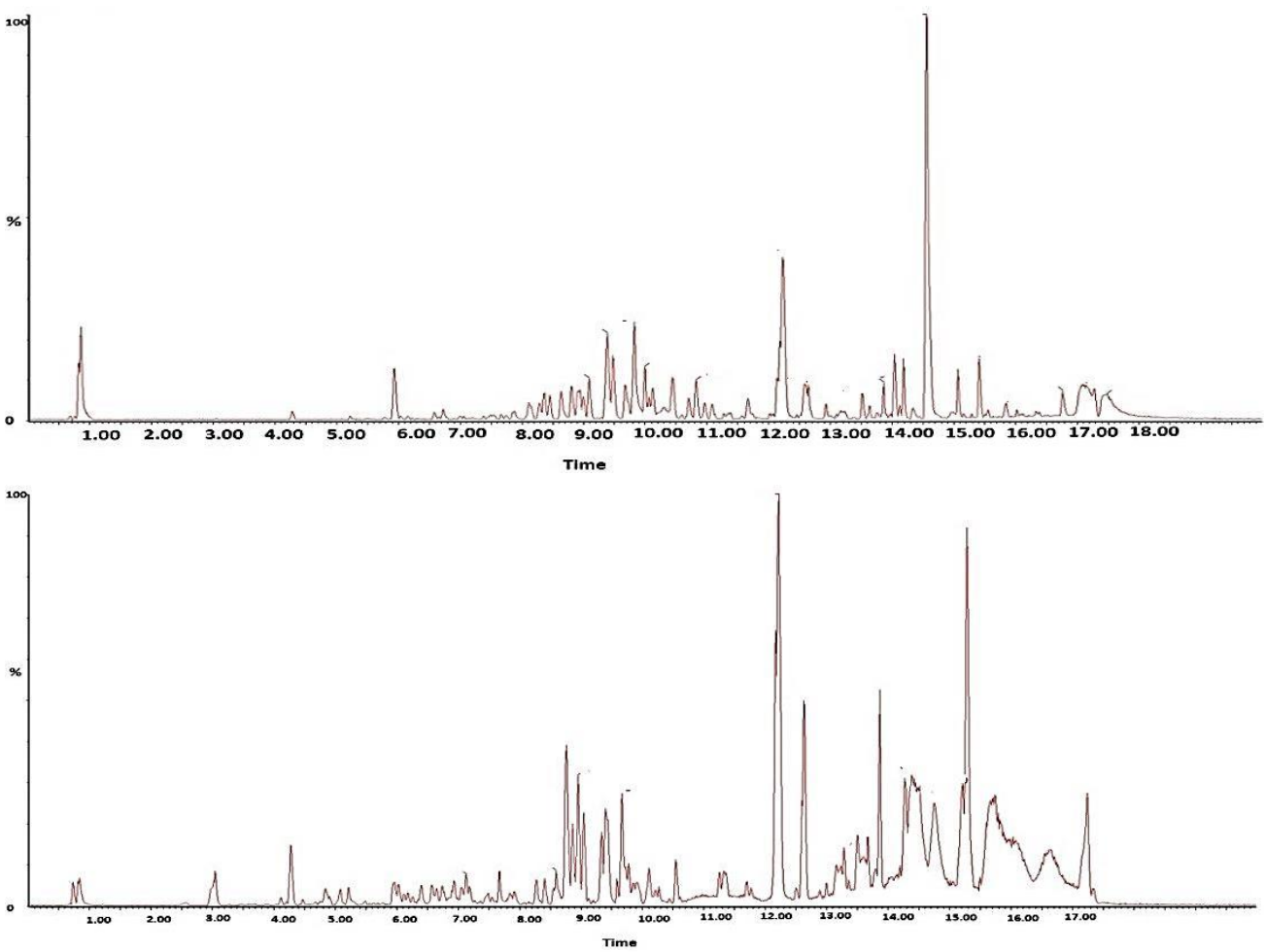


Fig. 1. A is a chromatogram of *Leonotis nepetifolia* in acetone (POSITIVE MODE), B is a chromatogram of *Leonotis nepetifolia* in acetone (NEGATIVE MODE).

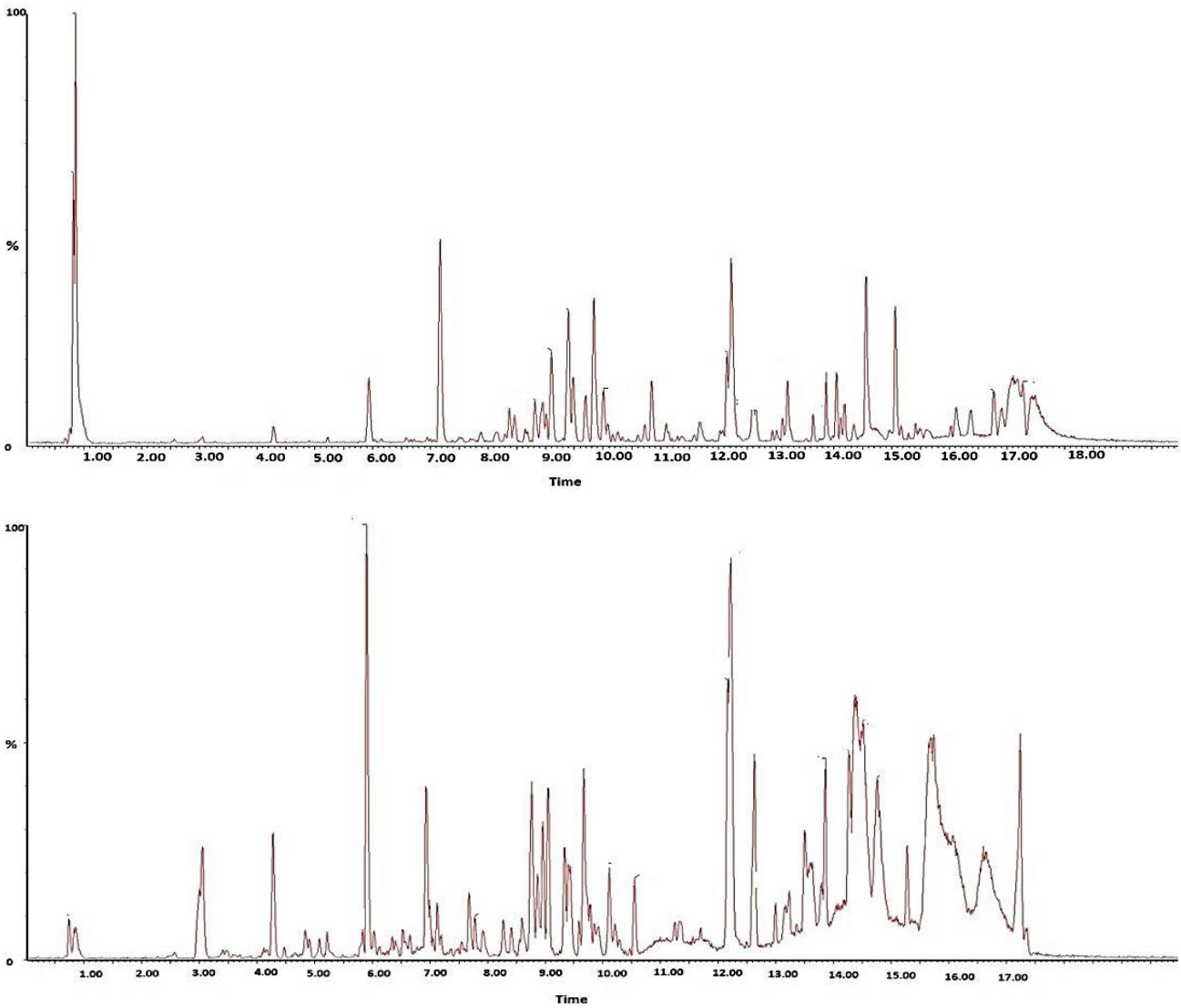


Fig. 2. A is a chromatogram of *Leonotis nepetifolia* in ethanol (POSITIVE MODE), B is a chromatogram of *Leonotis nepetifolia* in ethanol (NEGATIVE MODE).

Conclusion

The findings from this study suggest that *Leonotis nepetifolia* is a rich source of secondary metabolites. Flavonoids, alkaloids, terpenes, glycosides and fatty acids were the most common phytochemicals discovered in this study. According to the study's findings, ethanol made the best extract and had the most secondary metabolites detected. It was also revealed to be the most efficient solvent for extracting compounds with antibacterial activity. The presence of the aforementioned phytochemicals in this plant can be linked to its medicinal and antifungal potential. However, *In vitro* study of *Leonotis nepetifolia* is needed to validate its antifungal activities.

Acknowledgements

We greatly appreciate the National Research Foundation (NRF), for financial assistance in conducting this research. The authors thank the School of Biology and Environmental Science, the University of Mpumalanga for their immense contributions towards achieving this research work.

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(Received for publication 25 July 2022)