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South African Journal of Botany 172 (2024) 678–685

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Ethnomedicinal importance and antimicrobial activity of *Leonotis ocymifolia*

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ARTICLE INFO

Article History:
Received 23 December 2023
Revised 1 June 2024
Accepted 13 July 2024
Available online xxx

Edited by Dr. M Vambe

Keywords:
Leonotis ocymifolia
MIC
MBC
Time-kill
Toxicity
Candida

ABSTRACT

Species of the genus *Leonotis* (Lamiaceae) are commonly used in Africa as traditional herbal remedies for various ailments such as bronchial illness, epilepsy, stomach aches, and skin diseases. Most of these species are found in South Africa, and *Leonotis ocymifolia* (Burm.f.) Iwarsson (*klipdagga*) is one of them. The recorded medicinal uses of *L. ocymifolia* include treating diabetes, hypertension, anemia, nerve weakness, high blood pressure, poor blood circulation, rheumatism, snakebites, eczema, and several skin infections among others. Little is known about the antimicrobial activity. Therefore, this study aimed to investigate the antimicrobial potential of *L. ocymifolia* by reviewing the ethnomedicinal importance and antimicrobial potential (Minimum Inhibitory Concentration, Minimum bactericidal concentration, and Time-kill assessment) as well as the toxicity of *L. ocymifolia*'s extracts (DCM: Meth) and fractions (ethyl acetate, hexane, and butanol) against six bacteria and four *Candida* species. The results demonstrated that all the *Candida* pathogens tested were susceptible to the extracts, with moderate to noteworthy activity. The *n*-hexane fraction exhibited the highest antimicrobial activity against all tested pathogens, with mean MIC values between 0.06 – 0.25 mg/mL. Moderate activity was observed for the extracts against the bacteria tested, except for *Klebsiella pneumoniae*, where noteworthy activity (MIC value of 0.13 mg/mL) was found for the *n*-butanol fraction. A cidal effect on *C. glabrata* was recorded by the *n*-butanol fraction at 1xMIC (0.13 mg/mL) after 30 min of contact time and after 60 min at 1/2xMIC which is better than the positive control (Nystatin) where the cidal effect was only recorded after 4 h of contact time at 0.13 mg/mL. The hexane fraction was subjected to chemical analysis (gas chromatography-mass spectrometry) because of the noteworthy anticandidal activity. Nineteen compounds were identified with major compounds including methyl linolenate (33.31 %), *n*-hexadecanoic acid (21.32 %), phytol (21.35 %), and octadecenoic acid methyl ester (4.87 %). *Leonotis ocymifolia* has demonstrated promising antimicrobial properties, particularly against *Candida* species where the cidal effects link to the possible traditional use for *Candida*-acquired skin infections. The findings of this study, as determined by the brine shrimp lethality assay, indicate that usage of this plant extract poses minimal risk.

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1. Introduction

The genus *Leonotis* (Pers.) R.Br. is a member of the Lamiaceae family, commonly known as the mint family. Its notable features include fragrant leaves that are primarily utilized for medicinal purposes. This genus comprises of nine accepted African species, with three indigenous to South Africa [*Leonotis leonurus* (L.) R.Br., *L. nepetifolia* (L.) R.Br., and *L. ocymifolia* (Burm.f.) Iwarsson] (POWO, 2023). *Leonotis leonurus* (L.) R.Br.) is the most recognized species in this genus, commonly known as *wilde dagga* ("wild cannabis"), with a wide range of traditional uses (Nsuala et al., 2015).

Several other species share similarities with *L. leonurus* and could potentially have similar ethnobotanical uses. However, the traditional and modern uses of other species within the *Leonotis* genus, like *L. ocymifolia*, have not been extensively researched. *Leonotis ocymifolia* is a perennial woody shrub indigenous to South Africa (Fig. 1). It typically grows in rocky outcrops, riverbeds, and near evergreen forests (Iwarsson, 1985). Ethnobotanical data reveal that it has been traditionally used to prevent, treat, and mitigate diseases such as malaria. *Leonotis ocymifolia* can be taken orally or applied externally, depending on the medical condition. It is used for nerve weakness in the Cape Province of South Africa (Watt and Breyer-Brandwijk, 1962). According to Van Wyk et al. (2008), dried powdered leaves are taken as a snuff to treat headaches. The leaf infusion is taken as a drink to treat asthma. The pounded leaf infusion was reported as a

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https://doi.org/10.1016/j.sajb.2024.07.027
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Fig. 1. *Leonotis ocymifolia*: A) leaves and inflorescence (Montagu, Western Cape Province, South Africa), and B) habit (near Port St Johns, Eastern Cape Province, South Africa). Photographs by B.-E. Van Wyk.

remedy for colds. Other medicinal uses of *L. ocymifolia* have been documented in treating high blood pressure, poor blood circulation, rheumatism, and snakebites (Rattray and Van Wyk, 2021). Furthermore, *L. ocymifolia* is used as a decoction to treat stomach pains (Mashile et al., 2019). In developing countries, most citizens use traditional medicines to treat diarrhoea. Herbalists have widely used dried leaves of *L. ocymifolia* and honey to delay diarrhoea and reduce defecation (Ayele et al., 2021). The leaves of this plant species have been widely used for expelling parasites found in the intestines of animals, mainly in Oromia and the Bale Zone, Ethiopia (Yineger et al., 2007; Kefalew et al., 2015). The Ethiopian traditional healers use the leaf extracts medicinally for pain and inflammation due to the analgesic and anti-inflammatory effects (Alemu et al., 2018). Occasionally, it is used in treating ulcers and wounds, in addition to its application as an ascaricide and an anticancer agent (Habtemariam et al., 1994). This plant has also been reported to treat diabetes, hypertension, anaemia, eczema, and other skin infections (Asteraye, 2006). It has been reported as a remedy for neck ulcers, headaches, swelling, blackleg, hookworm, gout, and leishmaniasis (Ayele et al., 2021). The antifertility, antiemetic, and anti-plasmodial effects of *L. ocymifolia* have been reported (Tafesse et al., 2005; Lulekal et al., 2013; Teklu et al., 2020). Despite the numerous references to traditional uses against infectious diseases, the antimicrobial potential of *L. ocymifolia* has received limited research attention. Thus, this study focused on investigating the inhibitory and cidal efficacy of the extract and fractions from the leaf of *L. ocymifolia*. In addition, the toxicity was examined to determine a safety profile.

2. Materials and methods

2.1. Plant collection, extraction and solvent partitioning

Leonotis ocymifolia was collected at the Limpopo-Mpumalanga border, en route to Graskop (−24.56625, 30.74833) and identified by a taxonomist (Ben-Erik Van Wyk). A voucher specimen (RDR & BEVW 0032) was lodged in the Herbarium of the University of Johannesburg (JRAU). The leaves were air-dried, pulverized, and then milled using a Waring commercial blender to yield a fine powder. A portion (100 g) of the powdered sample was exhaustively extracted with dichloromethane (DCM): Methanol (50:50). The resulting extract was filtered (Whatman No. 1 filter paper) and subsequently concentrated at 45 °C using a rotary evaporator (Cole-Palmer, model SB-1100 Shanghai, China) to remove the organic solvent. The brown crude extract obtained was left in a fume hood to remove excess solvent. The dried organic extracts were stored in sealed, sterile glass bottles at room temperature for further analysis.

A portion (10 g) of the DCM: Methanol extract was reconstituted in 200 mL of sterile distilled water and dispensed into a clean separating funnel. Hexane (3 × 200 mL) was added, shaken mildly, and allowed to settle. The organic portion was separated, concentrated *in*

vacuo, and dried (2.00 g). In increasing polarity, the residual water-soluble portion was then consecutively partitioned with ethyl acetate (3 × 200 mL), and n-butanol (3 × 200 mL). The resulting organic portions were concentrated and dried under the fume hood. The yields obtained were 0.30 g (ethyl acetate) and 2.80 g (*n*-butanol) and refrigerated in air-tight containers.

2.2. Screening of Leonotis ocymifolia for antimicrobial activity

Samples were prepared by weighing the extract and fractions and adding a calculated solvent volume for a final starting concentration of 32 mg/mL for the minimum inhibitory concentration (MIC) assay. Culture and media preparation was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020). The choice of test organisms was based on recorded ethnobotanical uses. American Type Culture Collection (ATCC) microbial strains used in this study include three Gram-positive *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11178), *Streptococcus pyogenes* (ATCC 12344), three Gram-negative *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 13883), *Moraxella catarrhalis* (ATCC 23246), and four *Candida* strains; *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 90030), *Candida kruseii* (ATCC 14243), and *Candida tropicalis* (ATCC 14114).

The MICs were determined using the micro-plate dilution assay with tetrazolium violet reduction to indicate growth (Eloff, 1998; Oyedeji-Amusa et al. 2020). Sterilized Mueller Hinton broth and Tryptone Soya broth (100 mL) were aseptically added to each well of a 96-well microtiter plate for bacteria and *Candida*, respectively. Then, 100 mL of the respective plant extract (32 mg/mL) was added to the first row. Ciprofloxacin (0.01 mg/mL) was used as the positive control for the bacteria species, while Nystatin (1 mg/mL) was used as the positive control for the *Candida* species. Acetone and water were used as negative controls.

The mixture of the extract and broth in the first row was serially diluted down the column (16 to 0.0625 mg/mL). A volume of 100 mL of a 0.5 McFarland standardized culture suspension (1×10^8 colony forming units - CFU/mL) was added to each microtiter plate well. The plates were then sealed with a sterile adhesive sealing film (AEC Amersham) to prevent evaporation of the test sample, then incubated at 37 °C for 24 h and 48 h for bacteria and *Candida*, respectively. After incubation, 40 mL of 0.4 mg/mL *p*-iodonitrotetrazolium (INT) was added to each well. The microtiter plates were left at room temperature and when the colour changed in the control wells, the plates were analyzed to determine the MIC values. The MIC was interpreted as the first clear well, *i.e.*, the lowest sample concentration required to inhibit microbial growth. In the case of the *Candida* species, the plates were read after 24 h. The experiment was carried out in duplicate, and where variation was observed, a third replicate was undertaken. The MIC values ($\leq$ 0.16 mg/mL) considered to be noteworthy are marked in bold, while MIC values ($\geq 0.16 - < 1.00$ mg/mL) are considered to be moderate (Van Vuuren and Holl, 2017).

2.3. Minimum bactericidal concentration assay (MBC)

A standard method was employed to evaluate the minimum bactericidal concentrations of the *L. ocymifolia* extract and fractions (CLSI, 2020). Samples were taken from the MIC plates that exhibited microbial growth and were subsequently transferred onto newly prepared MHA and TSA plates for bacteria and *Candida*, respectively. The plates were incubated at 37 °C for 24 h in the case of bacteria spp., and 48 h for *Candida* spp. The minimum bactericidal concentration (MBC) was determined as the concentration of the extract that exhibited no discernible growth on a fresh set of agar plates.

2.4. Time-kill assay

The broth microdilution technique was adapted for the time-kill assay (Kurrimboccus et al., 2022). Sterile broth (100 μL) was added to the first row of a 96-well microtiter plate, while 100 μL of 0.90 % sodium chloride (NaCl) solution was added to the remaining rows. A 0.5 McFarland's standard of each pathogen was prepared to contain an approximate inoculum concentration of 1×10^8 CFU/mL, and 100 μL was transferred into the first row of the microtiter plate. Then, 100 μL of the *L. ocymifolia* extract (prepared at MIC and $^1/_2$ MIC concentration values) was transferred into the initial row of the microtiter plate. Subsequently, the contents of every well in the initial row, which comprised of broth, extract, and inoculum, were blended, and a volume of 100 μL was dispensed into the following well that contained 0.90% NaCl. Successive dilutions were then performed into 0.90% NaCl four times. A sample of 50 μL was taken from the dilution of every column at 0, 5, 10, 30, 60, 280, and 1440 mins and transferred onto agar plates (TSA) using a sterile spreader and incubated for 48 h. The logarithm of CFU/mL present in the original well against time was calculated (Eq. (1)) and was plotted onto a graph.

$$Log(CFU/mL) = 2log_{10}Colonycount \quad (1)$$

A known antifungal agent, Nystatin (0.13 mg/mL), where the same concentration was examined at the MIC of the extracts against *Candida glabrata* was used as a positive control to ensure microbial susceptibility. The negative control was the diluent acetone. A culture control of 100 μL of the prepared to a 0.5 McFarland's standard of the relevant pathogen reference strain was included to ensure viability and to obtain a growth curve for the pathogen studied. All assays were conducted in triplicate.

2.5. Toxicity

The toxicity screening was conducted using the brine shrimp lethality assay (BSLA) (Sarah et al., 2017). To create synthetic saltwater, 16 g of Tropic Marine sea salt was dissolved in 1 mL of deionized water. Then, 0.5 g of salt water and brine shrimp (*Artemia franciscana*) eggs (Ocean Nutrition) were introduced. The saltwater was aerated using a rotary pump (Kiho) and a steady supply of light for warming (220–240 V) to achieve a high hatch rate. The brine shrimp eggs were incubated for 18–24 h at 25 °C. After incubation, brine shrimp and seawater were transferred at an angle to a shallow plastic container, and a light source was placed on top for around 30 min, to gather a larger sample of brine shrimp. In a 48-well microtiter plate, 400 uL of saltwater containing 40–56 live brine shrimp was added to each well. Then, a 400 uL (2 mg/mL) plant sample of *L. ocymifolia* was added to each well after 30 min. The positive control was potassium dichromate (1.6 mg/mL), and the negative control was seawater and solvent of extract reconstitution (2 % dimethyl sulfoxide). Dead brine shrimp were counted after 24 and 48 h by observing plates under an Olympus light microscope at a magnification of 40X. Following a 48 h counting period, a lethal dose of acetic acid (Saarchem; 100 % (v/v); 50 mL) was added to each well. After 30 min, a final count was conducted to determine the percentage of mortality. According to Bussmann et al. (2011), a 50 % or higher mortality rate was deemed toxic. Experiments were carried out in triplicate.

2.6. Gas chromatography coupled with mass spectrometry (GC–MS) analysis

The dried hexane extract was reconstituted in 1 mL of chloroform and vortexed for 30 s before injection. Following this, 1 μL of the solution was injected into a Thermo Scientific™ TRACE™ 1300 gas chromatograph (Rodano, Milan, Italy), which was equipped with a ZB-5Ms capillary column (30 $m \times 0.25$ mm, 0.25 μm) and connected to a Thermo Scientific™ TSQ™ 8000 Triple Quadrupole mass

spectrometer (Austin, TX, USA). The GC–MS system incorporated a Triplus RSH Analytics PAL autosampler (Switzerland). Separation was carried out on a non-polar ZB-5Ms Zebron capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness) with helium as the carrier gas at a flow rate of 1 mL/min. The injector was kept at 250 °C, and 1 μL of the sample was injected in a 20:1 split mode. The oven temperature program began at 50 °C, held for 6 min, and then increased at 5 °C/min to 240 °C, with a total runtime of 46 min. The mass spectrometer operated in full scan mode with the ion source temperature set at 250 °C and the transfer line temperature at 280 °C. Electron impact ionization at 70 eV was used, scanning from *m/z* 35 to 600. Retention indices were determined under the same conditions using a homologous series of *n*-alkanes (C8–C24).

3. Data analyses

Data presentation was done using Microsoft Excel 365 and Graph Pad Prism 6 software (Diego, CA, USA). To identify The constituents of the hexane extract were identified by comparing their Retention Indices (RI) to Adams (2017) and their mass spectra to those in the NIST (v14)-MS library.

4. Results and discussion

4.1. Antimicrobial activity

The antimicrobial activity of the crude extract (DCM:Meth) and fractions (hexane, ethyl acetate, and butanol) of *L. ocymifolia* was studied against several micro-organisms. The results showed that the extract and fractions had varying degrees of activity against the tested organisms. The MIC and MBC values are represented in Table 1. *Leonotis ocymifolia* leaf extract and fractions showed noteworthy to moderate inhibitory activities against all *Candida* species studied, with MIC values ranging from 0.06 – 1.00 mg/mL. The *Candida* species demonstrated the highest susceptibility towards the hexane fraction (MIC value 0.06 mg/mL). All extracts showed moderate activity (MIC value range 0.25 – 1.00 mg/mL) against all bacteria species tested except for *Klebsiella pneumoniae* where the *n*-butanol fraction recorded noteworthy activity (MIC value 0.13 mg/mL). The MIC value for the positive control (nystatin) against the *Candida* species was 0.25 mg/mL, except for *Candida glabrata* where an MIC value of 0.13 mg/mL was recorded. The extract and fractions demonstrated comparable and, in some cases, better activity than the positive control (Nystatin). The MIC values varied between test pathogens for the positive controls (ciprofloxacin) against the bacteria species, with a range of 2.5 – 0.078 μg/mL, showing that the conventional antimicrobials were responsive. On the other hand, the negative controls (acetone and water) did not inhibit the growth of the test pathogens, thus demonstrating the suitability of the solvent used.

Vagionas et al. (2007) reported MIC values ranging from 2.00 to 4.85 mg/mL for *L. ocymifolia* essential oil against *C. albicans, C. tropicalis,* and *C glabrata,* while Bekele et al. (2014), reported an MIC value of 250 mg/mL for the methanol leaf extract of *L. ocymifolia* against *C. albicans*. The extract and fractions tested showed varied cidal effects depending on the pathogen studied. Cidal effects (MBC value range 0.13 – 1.00 mg/mL) were noted for *Candida glabrata* against the extract and fractions tested. The extract and fractions recorded a cidal effect against *Staphylococcus aureus* and *Streptococcus pyogenes* (Table 1). The MBC value for the positive control (Nystatin) against the *Candida* species was 0.25 mg/mL, except for *Candida albicans,* where no cidal effect was recorded. The MBC values recorded for the positive control (ciprofloxacin) against the bacteria species were 2.5 μg/mL and 0.3 μg/mL, except for *E. coli,* where no cidal effect was recorded.

The noteworthy MIC value and cidal effect recorded against *K. pneumonia* are interesting, considering that *L. ocymifolia* has traditionally been used for treating urinary tract and bloodstream infections. Also, the traditional uses of this plant against skin infections can be justified by the noteworthy MIC values and cidal effect exhibited by the extracts against all *Candida* species tested. *Candida* is a fungal strain that can potentially induce infections in various body areas, including the skin. Under typical circumstances, the skin may harbour minimal quantities of this yeast. However, under immunocompromised conditions, the yeast starts to undergo multiplication, resulting in excessive proliferation. HIV-positive patients may have an increased susceptibility to *Candida* infections (Owotade et al., 2016).

Furthermore, *C. albicans* remains the most predominant infectious-causing pathogen in both adult and pediatric patients largely due to the biofilm formation on biological and artificial surfaces

Table 1
Minimum inhibitory concentration (MIC) (mg/mL) and minimum bactericidal concentration (MBC) (mg/mL) of extract and fractions of L. *ocymifolia* extracts against selected pathogens.

Pathogen classifica-tion	Organisms	MIC (mg/mL)					MBC (mg/mL)				
		DCM:Meth	Hex	Ethyl	But	Positive control	DCM:Meth	Hex	Ethyl	But	Positive control
Candida	*Candida albicans* (ATCC 10231)	0.50	**0.06**	0.25	**0.06**	0.25	N/I	2.00	N/I	1.00	N/I
	Candida glabrata (ATCC 90030)	**0.06**	**0.06**	**0.13**	**0.13**	0.13	0.25	0.50	1.00	0.13	0.25
	Candida kruseii (ATCC 14243)	1.00	**0.06**	0.50	0.25	0.25	8.00	N/I	N/I	N/I	0.25
	Candida tropicalis (ATCC 14114)	**0.13**	**0.06**	**0.13**	**0.13**	0.25	2.00	1.00	N/I	N/I	0.25
Gram-negative	*Escherichia coli* (ATCC 8739)	0.50	0.25	0.25	0.25	0.25×10^{-2}	4.00	N/I	2.00	2.00	N/I
	Klebsiella pneumoniae (ATCC 13883)	1.00	0.25	0.25	**0.13**	0.78×10^{-4}	N/I	4.00	4.00	2.00	0.31×10^{-3}
	Morexella catarrhalis (ATCC 23246)	0.50	0.50	0.25	0.25	0.25×10^{-2}	N/I	N/I	N/I	4.00	0.25×10^{-2}
Gram-positive	*Staphylococcus aureus* (ATCC 25923)	0.50	0.25	1.00	0.25	0.13×10^{-2}	2.00	4.00	2.00	N/I	0.25×10^{-2}
	Bacillus cereus (ATCC 11778)	0.25	0.25	0.25	0.25	0.25×10^{-4}	4.00	N/I	4.00	N/I	N/I
	Streptococcus pyogenes (ATCC 12344)	0.25	0.25	0.25	0.25	0.13×10^{-2}	2.00	4.00	2.00	N/I	0.25×10^{-2}

DCM= Dichloromethane; Meth= Methanol; Hex= Hexane fraction; Ethyl= Ethyl acetate fraction; But = Butanol fraction; N/*I*= No Inhibition at highest concentration tested; MIC values ≤ 0.16 mg/mL= Noteworthy activity and indicated in bold; MIC values ≥ 0.16 – < 1.00 mg/mL = Moderate activity.

(Wang et al., 2022). These biofilms play a role as essential virulence factors that cause various pathologies. Based on this, there is a growing need for the development of novel and effective antifungal agents (Wang et al., 2022).

4.2. Time-kill assessment

As the *Candida* species demonstrated the lowest MIC values and most promising antimicrobial activity of the extracts, a time-kill assay was undertaken to determine the cidal response to time. While all three *Candida* species demonstrated noteworthy activities, *C. glabrata* demonstrated the lowest MIC values of the three studied across all the extracts. Furthermore, cidal activity (MBC) was observed for the DCM/methanol extract as well as the hexane and butanol fractions of *L. ocymifolia* which were thus further investigated in the time-kill assay.

The time-kill plots are shown in Figs. 2A and B. For the butanol fraction, a 74% reduction in the colony-forming units (CFUs) was recorded after 10 min of contact time with *C. glabrata* at 1 × MIC (0.13 mg/mL), and a cidal effect was recorded after 30 min of contact time at the same concentration. When the concentration (butanol fraction) was decreased to 1/2 × MIC (0.06 mg/mL), a 77% reduction in CFUs was recorded after 30 min of contact time and a cidal effect recorded after 60 min (1 h) of contact time. This activity recorded by the butanol fraction is favorably comparable to the positive control (Nystatin), where a 74% reduction in CFUs was recorded after 60 min (1 h) of contact time with *C. glabrata*. A cidal effect was only recorded after 240 min (4 h) of contact time (Fig. 2A).

For the hexane extract (Fig. 2B), a cidal effect was recorded after 5 min of contact time with *C. glabrata* at 1 × MIC (0.06 mg/mL). When the concentration (hexane fraction) was decreased to 1/2 × MIC (0.03 mg/mL), a 77% reduction in CFUs was recorded after 10 min of contact time and a total cidal effect after 30 min. This activity recorded by the butanol extract is comparable to the positive control (Nystatin), where a 74% reduction in colony forming unit was recorded after 60 min (1 h) of contact time with *C. glabrata* and a total cidal effect was only recorded after 240 min (4 h) of contact time (Fig. 2B).

As expected, the negative control, (solvent acetone: water) showed no activity and displayed comparable growth to the culture control (Figs. 2A and B).

4.3. Toxicity

The average percentage mortalities (n = 3) of brine shrimp exposed to the leaf extract and fractions of L. *ocymifolia* are represented in Table 2. All percentage mortality rates observed were below 50%, except for the positive control (potassium dichromate), where 83.45% mortality was recorded after 24 h and 100% mortality after 48 h. Hence, all the extracts investigated were considered non-toxic (Bussmann et al., 2011). *Leonotis ocymifolia* was deemed safe to use at a concentration of 2 mg/mL, as evidenced by the mortality rate

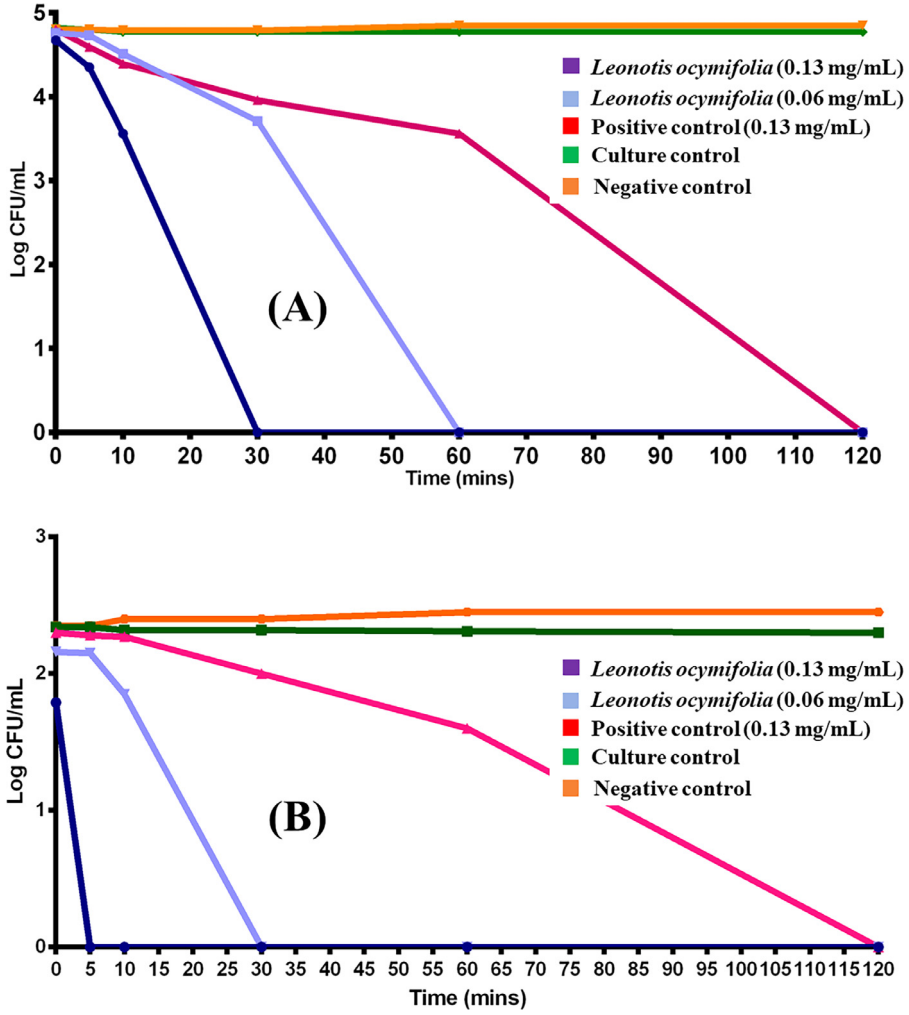


Fig. 2. Time-kill rate of *Leonotis ocymifolia* against *Candida glabrata*: A) butanol extract; and B) hexane extract.

Table 2
Percentage mortality of brine shrimp after exposure to different extracts (2 mg/mL) of *Leonotis ocymifolia.*

Extract	Percentage mortality (n = 3)	
	24 h	48 h
DCM: Meth	8.33	8.45
Hexane extract	9.17	10.00
Butanol extract	5.77	5.39
Ethylacetate extract	10.00	10.65
Potassium dichromate	83.45	100
Dimethyl sulfoxide	2.63	2.63
Seawater	2.38	7.14

recorded for the extracts in this study. The toxicity result in this study is in line with previous studies on the toxicity of *L. ocymifolia*. The *in vivo* toxicity study of *L. ocymifolia* has been reported in female albino rats following the OECD guidelines, and the methanol leaf extracts were reported to be safe according to the LD_{50} value recorded (Alemu et al., 2018; Teklu et al., 2020).

4.4. GC–MS analysis of the hexane fraction

The chemistry of the genus *Leonotis* has been well-studied with compounds such as labdane terpenoids being reported. Known compounds of this class include marrubiin (Rivett, 1964), compounds X and Y (Kaplan and Rivett, 1968), and leonurenones A–C (He et al., 2012). Based on the noteworthy cidal activity of the hexane fraction, chemical analysis was performed to understand better the compounds that may be responsible for the noteworthy anti-candidal activity. Gas chromatography coupled with mass spectrometry (GC–MS) showed several peaks of interest (Fig. 3), which could be identified by their mass spectra (Table 3). Major and minor compounds include phytol (21.4 %) and fatty acids and their esters such as *n*-hexadecanoic acid (21.3 %), methyl linolenate (33.3 %), and octadecenoic acid methyl ester (4.9 %). Similar compounds have been identified in flower bud hexane fractions of the closely related species *L. nepetifolia* (L.) R.Br. by GC–MS (Nagaraja et al., 2023). Nagaraja et al. (2023) identified 24 compounds from the hexane extract where the major compounds included pentadecanoic acid, eicosane and tetratriacontane. Furthermore, the antimicrobial activity of the extract was tested on *Staphylococcus aureus, E. coli, C. albicans* and *C. glabrata*, with *S. aureus* and *C. glabrata* exhibiting sensitivity towards the hexane extract.

Table 3
Chemical compounds in hexane extracts of *Leonotis ocymifolia* identified by Gas Chromatography-Mass Spectrometry.

RT	RRI	Area (%)	Compound	Compound class
28.17	1521	0.11	γ-muurolene	Sesquiterpene
28.51	1535	0.78	dihydroactinidiolide	Terpene
29.70	1584	0.16	spathulenol	Sesquiterpene
29.84	1590	0.21	caryophyllene oxide	Sesquiterpene
33.71	1761	0.16	tetradecanoic acid	Long-chain fatty acid
35.39	1841	0.54	neophytadiene	Diterpene
37.15	1927	2.98	hexadecanoic acid, methyl ester	Fatty acid methyl ester
37.47	1943	0.28	palmitoleic acid	Fatty acid
37.97	1968	**21.32**	*n*-hexadecanoic acid	Fatty acid
40.05	2077	0.15	(9Z,12Z,15Z)-(E)–3,7-dimethylocta-2,6-dien-1-yl octadeca-9,12,15-trienoate	Fatty acid ester
40.41	2096	1.28	9,12-octadecadienoic acid, methyl ester, (E,E)-	Fatty acid methyl ester
40.53	2102	4.87	octadecenoic acid, methyl ester	Fatty acid methyl ester
40.77	2116	**21.35**	phytol	Diterpene
40.99	2128	0.64	methyl stearate	Fatty acid methyl ester
41.37	2149	**33.31**	methyl linolenate	Fatty acid methyl ester
41.49	2155	2.03	1-(3,7-dimethyl-1-octenyl)-cyclopropanol	Fatty acid ester
41.66	2165	3.22	octadecanoic acid	Long-chain fatty acid
42.66	2221	0.12	phytol acetate	Terpenoid ester
44.03	2300	0.11	eicosane	Alkane
		93.65	% Identification	

RT = Retention Time; RRI = Relative Retention Index.

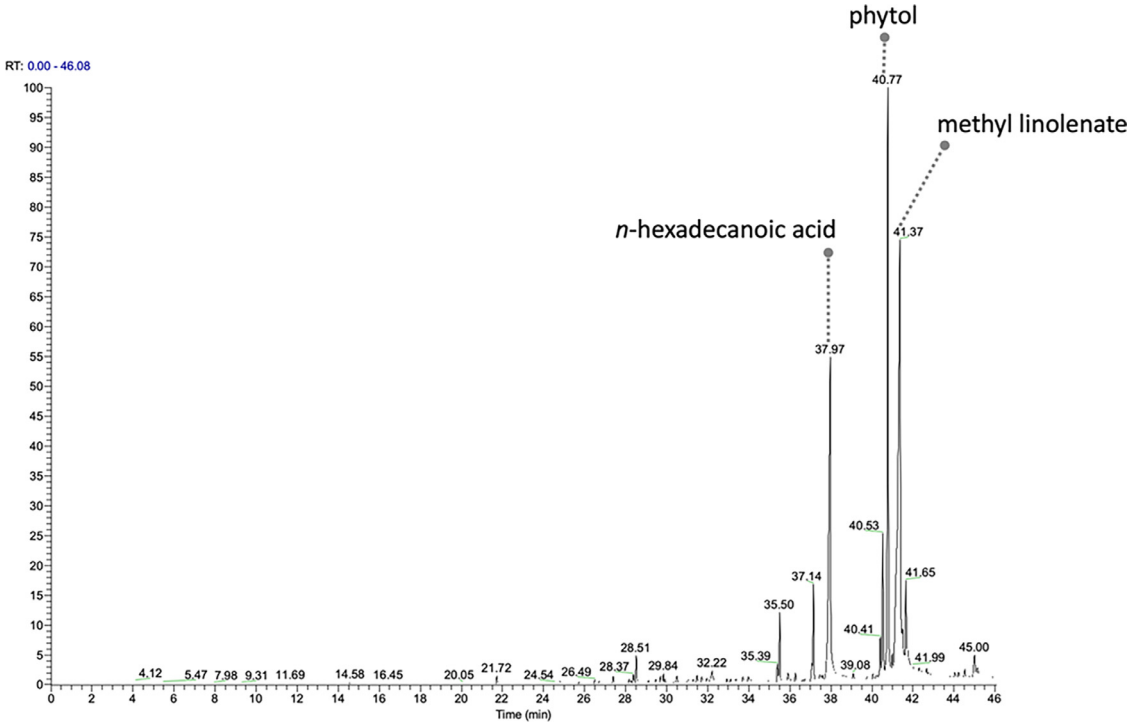


Fig. 3. GC–MS chromatogram generated for the hexane fraction of *Leonotis ocymifolia*. Major compound names are indicated above the corresponding peaks.

Phytol, a constituent of the chlorophyll molecule found in virtually all photosynthetic organisms, is utilised in the fragrance industry and is recognised for its diverse biological effects. Additionally, phytol has demonstrated a broad spectrum of antimicrobial activity, encompassing efficacy against eukaryotic microorganisms such as *Candida albicans* (Islam et al., 2018).

Several studies have demonstrated the noteworthy antifungal activity of fatty acids against *Candida albicans*. For example, Lee et al. (2021) reported the efficacy of medium-chain fatty acids, while Shareck et al. (2011) documented the inhibition of *C. albicans* hyphal growth by linoleic acid. These findings suggest that fatty acids could be promising candidates for antifungal therapies. The combination of phytol, fatty acids, and fatty acid esters may contribute to the significant cidal activity observed in the hexane fraction of *L. ocymifolia* demonstrated by this study.

5. Conclusions

Leonotis ocymifolia has demonstrated promising antimicrobial properties, particularly against *Candida* species, where the cidal effects link to the possible traditional use for *Candida*-acquired skin infections. This noteworthy activity could likely be ascribed to compounds such as fatty acids, fatty acid esters and phytol – all of which have documented anti-candidal activity. Furthermore, *L. ocymifolia* demonstrated no toxicity, providing additional rationale for more scientific explorations of its antimicrobial effects. The species seems to have considerable potential as a starting point for the development of an anticandidal agent of natural origin.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Mariam O. Oyedeji-Amusa: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Sandy F. Van Vuuren:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Ryan D. Rattray:** Formal analysis, Investigation, Methodology, Software, Writing – review & editing. **Ben-Erik Van Wyk:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Acknowledgements

We thank Phumzile Moerane for microbiological technical support, Thato Moloi for preparing the extracts, and Lucky Mokwena and William Arries for assistance with the GC–MS.

Funding sources

This work was supported by the National Research Foundation of South Africa (Grant Nos. 138484 and 84442) and by the University of the Witwatersrand (RINC funding).

Ethical approval statement

Not applicable.

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