




Antifungal activity and toxicity of bioactive compounds isolated from the leaf of *Ximenia caffra* Sond. var. *natalensis*

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Background: Based on high frequency index, *Ximenia caffra* Sond. var. *natalensis* was selected for further phytochemical investigation and biological assays.

Aim: The study aimed to isolate the active antifungal compounds from the leaves of *X. caffra* var. *natalensis*.

Setting: The ethnobotanical study was conducted in Aganang Local Municipality, Capricorn District.

Methods: Acetone extract was partitioned five times with hexane, chloroform, ethyl acetate, butanol and water, respectively. Fractions were screened for antifungal activity against *Candida albicans* using the microplate method and bioautography assays. The structures of isolated compounds were identified using nuclear magnetic resonance (NMR) and mass spectrometry. Cytotoxicity of isolated compounds was determined using 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Results: Bioassay-guided fractionation of the ethyl acetate fraction led to the isolation of four compounds, out of which only two were identified. Compound 1 was identified as epigallocatechin gallate, and Compound 3 was confirmed as kaempferol-3-O-rhamnoside. Epigallocatechin gallate exhibited moderate antifungal activity with minimum inhibitory concentration (MIC) of 0.5mg/mL and less toxic to the cells with LC50 = 32.32 µg/mL.

Conclusion: The antifungal activity and cytotoxicity of isolated compounds validate the use of *X. caffra* Sond. var. *natalensis* in combating oral candidiasis.

Contribution: The results have shown the potential bioactivity of *X. caffra* Sond. var. *natalensis* in the treatment of oral candidiasis.

Keywords: oral candidiasis; antifungal activity; cytotoxicity; minimum inhibitory concentration (MIC); *Ximenia caffra* Sond. var. *natalensis*.

Introduction

Ximenia caffra (*X. caffra*) Sond. var. *natalensis* is a shrub or small tree that grows a little over 2 m long and belongs to the Olacaceae family (Hyde et al. 2023). It is a terrestrial plant distributed along woodlands and rocky outcrops of Limpopo, Mpumalanga and Kwazulu-Natal provinces of South Africa (Foden & Potter 2005). *X. caffra* Sond. var. *natalensis* bears oval, bright red edible fruit and tastes sour (Van Wyk & Van Wyk 1997). Previous studies indicate the plant is used for the treatment of diarrhoea, abdominal pains, eye inflammation and sores (Baloyi & Reynolds 2004). As part of our search for medicinal plant species used for the treatment of oral candidiasis in Aganang Local Municipality, Limpopo Province of South Africa (Tlaamela 2019), we report the activity and structure of compounds isolated from the leaves of *X. caffra* Sond. var. *natalensis*. *X. caffra* Sond. var. *natalensis* was selected for this purpose because it was among the frequently used plant species and also demonstrated stronger antifungal activity against *Candida albicans*. Oral candidiasis is among the well-known opportunistic diseases affecting most people, including immune-compromised patients and human immunodeficiency virus (HIV) patients (Ellepola & Samaranyake 2000).

Oral candidiasis is characterised by the overgrowth of *Candida* species in the epithelium of the oral mucosa. An increase in oral candidiasis incidences, particularly in developing countries, has

become a major public health concern (Sanguinetti, Posteraro & Lass-Flörl 2015; Whibley & Gaffen 2015). Ten million cases of oral candidiasis and two million cases of esophageal candidiasis are reported annually in people living with HIV (Fanou et al. 2020). Hence, several *Candida* species including *C. albicans* have become the focus of several studies (Fanou et al. 2020; Masevhe, McGaw & Eloff 2015). Commercially available drugs for oral candidiasis include: amphotericin B, nystatin, itraconazole, clotrimazole, miconazole, echinocandins and fluconazole (Akpan & Morgan 2002). However, because of prolonged misuse of these drugs, the pharmaceutical industry is currently faced with challenges of drug resistance (Sanguinetti et al. 2015). Furthermore, some drugs are expensive and have side effects. These challenges necessitate a need to search for and evaluate alternative drugs that are effective, non-toxic and accessible.

Plants are among the most important sources of potentially valuable new drugs (Koné et al. 2004). Medicinal plant products may be used as crude extracts, or active compounds may be isolated and their chemical structures are explored as starting material or lead compounds during the design and discovery of new drugs including antifungal agents (Ahmed 2016). Medicinal plants synthesise a wide range of secondary metabolites such as phenols, terpenoids, flavonoids, alkaloids, steroids, tannins, saponins and other chemical compounds that perform important biological functions (Erhabor et al. 2021; Mkhonto et al. 2023). These secondary metabolites possess various pharmacological properties and activities such as antioxidant, antimicrobial, anticancer and antidiabetics (Pedrollo et al. 2016; Seepe et al. 2021). Plant extracts are composed of a variety of different chemical constituents with different polarities that have multiple physiological effects. Therefore, the isolation of compounds is important in knowing and assessing the potential of individual bioactive compounds. Isolated active compounds from plants with a long history of use are likely to be safer than synthetic compounds (Ginovyanyan, Petrosyan & Trchounian 2017).

Ethnobotanical studies play an important role in documenting important plant species with potential biological activities (Tlaamela & Mahlo 2021). This is supported by the discovery of compounds through plant-based ethnobotanical surveys (Mbunde et al. 2017). The availability of plant-based medicine may help alleviate antifungal drug resistance while mitigating the side effects associated with synthetic antifungals. Moreover, plant-extractable compounds have gained much attention in conventional medicine (Rezk et al. 2015).

Cytotoxicity testing is important as a first step in validating the safety of potent medicinal plant extracts and bioactive compounds. It detects loss of membrane integrity associated with cell death (Niles, Moravec & Riss 2009). The severity of the toxic effect on plants may depend on the amount consumed, the growth stage of the plant, administration mode (Botha & Penrith 2008), the solubility of the toxin in

body fluids or the frequency of intoxication (Ndhlala et al. 2013). The cytotoxic activity of medicinal plants is necessary to test if these compounds are harmful to humans. The current study focuses on the antifungal activity and cytotoxic effects of compounds isolated from the leaves of *X. caffra* var. *natalensis*.

Research methods and design

Plant collection and identification

Leaves of *X. caffra* var. *natalensis* were collected from Boratapelo village, Aganang Local Municipality (Figure 1), and allowed to dry for 3 weeks. Ethical clearance was granted by the Turfloop Research Ethics Committee (TREC/385/2017:PG) prior to the commencement of the study. Literature and the University of Limpopo herbarium were used to identify the plant species by their scientific names. The voucher specimen (DK 01) of the plant species was prepared and deposited at the herbarium.

Plant extraction

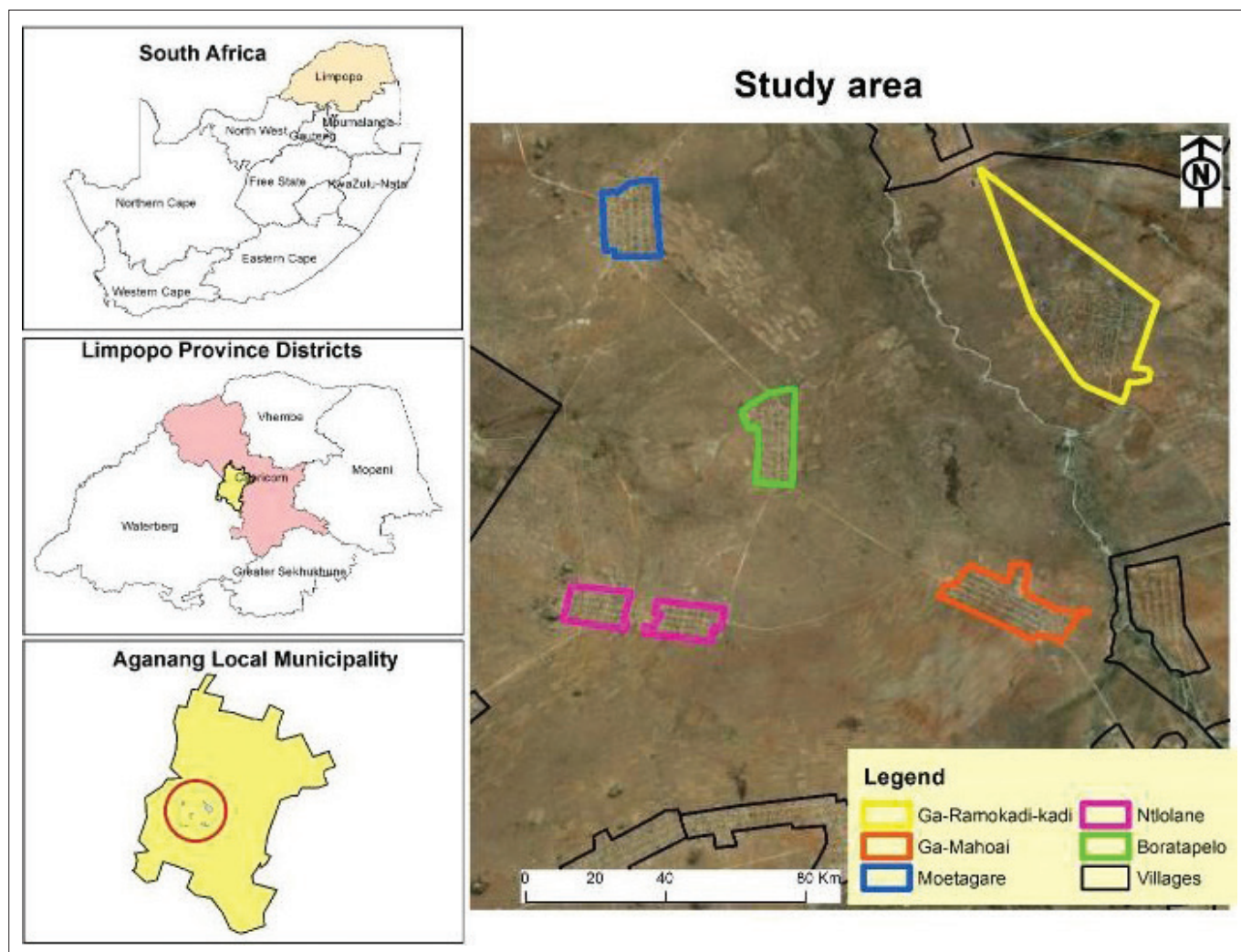
The plant materials were ground to a fine powder using a laboratory grinding mill (Macsalab 200 LAB, Eriez) and stored in airtight bottles in the dark until extraction. Finely ground material (828.38 g) was extracted with acetone (1:10) (Eloff 1998a). The plant extract was shaken vigorously for 30 min, filtered, and then allowed to evaporate. The resulting filtrate was dried under reduced pressure at 40°C using a rotavapor (Büchi rotary evaporator), and the extracts were transferred into a pre-weighed glass jar. The extracts were placed under a stream of cold air to allow complete evaporation of the solvents. The plant materials were washed three times with acetone and the crude extracts were combined.

Phytochemical analysis

Chemical constituents of the extracts were analysed using aluminium-backed Thin Layer Chromatography (TLC) plates that were developed with three eluent systems: Chloroform: ethyl acetate: formic acid: 20:16:4 (CEF), ethyl acetate: methanol: water: 40:5.4:4 (EMW), and benzene: ethanol: ammonia hydroxide: 90:10:1 (BEA) (Kotze & Eloff 2002). Ten microliters of plant extracts were loaded on TLC plates. Chemical components were visualised under visible and ultraviolet light (254 nm and 360 nm, Camac Universal Ultraviolet [UV] lamp TL-600). For chemical compounds not visible under UV light, vanillin-sulphuric acid spray reagent was used for detection (Stahl 1962).

Fungal strains and inoculum quantification

Candida albicans (American Type Culture Collection [ATCC 10231]) was obtained from the culture collection of the Department of Veterinary Tropical Diseases at the University of Pretoria. This opportunistic fungal pathogen causes oral candidiasis in humans. The haemocytometer cell-counting method described by (Aberkane et al. 2002) with some modifications was used to quantify fungi by counting the



Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

FIGURE 1: Map of Aganang Local Municipality.

number of cells for each fungal culture prior to conducting the assays. The inoculum of each isolate was prepared by growing the fungus on Sabouraud Dextrose (SD) agar slants for 24 h at 37°C. The final inoculum concentration was adjusted to approximately 1.0×10^6 cells/mL.

Bioautography

Thin layer chromatography plates were loaded with 10 μ L of each plant extract. The plates were developed in different eluent solvent systems such as EMW, CEF and BEA. The chromatograms were dried under a stream of air to evaporate solvents. The developed plates were sprayed with an overnight culture of *C. albicans* until they were completely wet. The plates were incubated at 37°C in a clean humidified chamber overnight, and further sprayed with a solution of p-iodonitrotetrazolium (INT) violet and incubated for 2–6 h for fungal growth. White areas were indicated where reduction of INT to the coloured formazan did not take place because of the presence of compounds that inhibited the growth of the fungi. The active compounds visible on TLC chromatograms were targeted for bioassay-guided fractionation.

Solvent-solvent fractionation

Solvent-solvent fractionation was conducted according to Mahlo, McGaw and Eloff (2013) with some modifications. The acetone extract (95.14 g) of *X. caffra* var. *natalensis* was partitioned five times with hexane, chloroform, ethyl acetate, butanol and water, respectively. The crude extract of *X. caffra* var. *natalensis* was dissolved in 800 mL hexane and mixed with an equal amount of distilled water in a 2 L separating funnel. After the separation of the two layers, the bottom layer was collected to yield the aqueous fraction. The process was repeated three times by extracting the water fraction with chloroform. After separation, the bottom layer was collected to yield the chloroform fraction and the residue was further mixed with an equal amount of ethyl acetate and allowed to separate into two layers. The ethyl acetate fraction was collected and the residue was further mixed with an equal amount of butanol and allowed to separate. After separation, the top layer was collected yielding a butanol fraction and the bottom layer yielded a water fraction. The fractions were collected in pre-weighed jars and evaporated to dryness at 40°C under reduced pressure using a Büchi Rotavapor.

Column chromatography

The ethyl acetate fraction (15.15 g) obtained from solvent-solvent fractionation was dissolved in a minimal amount of ethyl acetate and mixed with silica gel (Mahlo et al. 2013). This fraction had shown good antifungal activity against *C. albicans* as compared to other fractions. The column was initially eluted with 100:0 of DCM: MeOH; and the polarity of the mixture was sequentially increased to 0:100. Fractions were collected in conical flasks (100 mL). The fractions were evaporated under reduced pressure and subjected to TLC analysis using EMW [40:5.4:4]. The fractions with similar spots were pooled together, resulting in 10 fractions, and evaporated under reduced pressure. Fractions 37–38 and 39–42 obtained from the first column (column i) were loaded on TLC plates and developed using EMW [40:5.4:4]. The fractions were tested for antifungal activity against *C. albicans* using the microdilution method and bioautography assay.

Antifungal assay

The microplate method with some modifications was used to determine the minimal inhibitory concentration (MIC) values of the selected fractions (Eloff 1998b). The plant extracts (100 µL) and pure compound were serially diluted (50%) with distilled water in 96-well microtiter plates and fungal culture (100 µL) was added to each well. The plant extracts and isolated compounds were tested in triplicate in each assay. Amphotericin B was used as a positive control and 100% acetone as a negative control. As an indicator of growth, 40 µL p-iodonitrotetrazolium violet (INT) of 0.2 mg/mL in water was added to the microplate wells. The covered plates were incubated at 37°C for 24 to 72 h.

Preparative thin layer chromatography

Fractions 39–42 were combined and removed under a stream of cold air at room temperature. Phytochemical analysis of this fraction contained two compounds, and they were further separated by preparatory thin-layer chromatography. Furthermore, the fraction was dissolved in methanol, loaded on a preparative TLC (pTLC) plate and developed in EMW. The chromatogram was visualised under UV light. Visible compounds were scraped with a clean blade into glass jars and dissolved in 10% MeOH: DCM or 100% methanol. They were then filtered into pre-weighed vials, evaporated and subjected to TLC analysis developed in EMW, yielding Compound 1 and Compound 3. Compound 3 was repeatedly subjected to pTLC for purification.

Sephadex LH-20 column chromatography

Fractions 37–38 obtained from column (i) were further purified using Sephadex LH-20 column chromatography (column ii). The fraction was dissolved in 1 mL of methanol and loaded into a Sephadex-containing glass column. Methanol (100%) was used to elute the column until the stationary phase was clear (Zhang et al. 2014). Fractions were evaporated under reduced pressure and subjected to phytochemical analysis. Fractions containing the same spots

were combined. Fractions F14–16 of column ii were further separated by Sephadex LH-20 column following the same procedure (column iii). Fractions of 20 mL were collected. TLC chromatograms of these fractions contained some impurities. Fractions 1–2 were pooled together and purified further using pTLC analysis to yield compounds 3 and 4.

Structure elucidation

Nuclear magnetic resonance (NMR) (1D and 2D) spectroscopic and mass spectrometry data were used for the identification of the isolated compounds. ¹H-NMR, ¹H-¹H COSY and ¹³C-NMR experimental data were acquired on a 400 MHz NMR spectrometer (Bruker Avance III 400 MHz). HPLC-HR-ESI-MS was performed on a Waters Acquity Ultra Performance Liquid Chromatography (UPLC®) system hyphenated to a quadrupole-time-of-flight (QTOF) instrument. The structures were interpreted with the use of published data.

Cytotoxicity assay

African green monkey kidney (Vero) cells were used to test the cytotoxicity of isolated compounds using the tetrazolium-based colorimetric (MTT) assay (Mosmann 1983). Cells were cultured in Minimal Essential Medium (MEM) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). Cells were then trypsinized using trypsin-EDTA (Sigma) and the density was adjusted to 5 × 10⁴ cells/mL. Cell suspension (200 µL) was added to each well of 96-well microtiter plates. The plates were incubated for 24 h at 37°C in a 5% CO₂ incubator until the cells were in the exponential phase of growth. The MEM was aspirated from the cells, which were then washed with 150 µL phosphate-buffered saline (PBS, Whitehead Scientific) before the addition of 200 µL of test compound at differing concentrations in quadruplicate. Stock solutions of isolated compounds (20 mg/mL) were prepared by dissolving them in dimethyl sulfoxide (DMSO). Appropriate dilutions of each extract and isolated compounds were prepared in a growth medium and added to the cells. The viable cell growth after 120 h incubation with the isolated compound was determined using the tetrazolium-based colorimetric assay (3-[4,5-dimethylthiazol]-2,5-diphenyl tetrazolium bromide (MTT) (Mosmann 1983). Doxorubicin chloride (Pfizer Laboratories) was used as a positive control and untreated cells as a negative control. The absorbance was measured on a microplate reader (Biotek, Synergy HT) at a wavelength of 540 nm and a reference wavelength of 630 nm. The procedure was repeated three times on different occasions.

Results and discussion

Finely ground material (828.38 g) was extracted with acetone, yielding acetone crude extract (95.14 g). This extract was fractionated five times with hexane, chloroform, ethyl acetate, butanol and water, respectively. The quantities of five yielded fractions were hexane (3.46 g), chloroform (2.8 g), ethyl acetate (15.51 g), butanol (39.64 g) and water (9.05 g). The highest quantity of plant material was extracted with butanol,

followed by ethyl acetate fraction. The lowest fraction was obtained from chloroform.

The obtained fractions were determined for antifungal activity against *C. albicans* using the microplate method. Strong antifungal activity was exhibited by butanol and ethyl acetate fractions, both with a MIC value of 0.08 mg/mL (Table 1). Similar findings for butanol fractions were reported for *Breynia salicina* against *Penicillium expansum*, *Penicillium janthinellum* and *Fusarium oxysporum* (Mahlo et al. 2013) and *Coccoloba cowellii* against *C. albicans* (Méndez et al. 2021). Strong antifungal activity of ethyl acetate fractions was reported for *C. cowellii* against *C. albicans* (Méndez et al. 2021). Moderate activity was observed in the chloroform, aqueous and hexane fractions. However, Méndez et al. (2021) reported good antifungal activity of hexane fraction of *C. cowellii* leaves against *C. albicans*. Chloroform fraction of *B. salicina* had strong antifungal activity against *P. expansum*, *P. janthinellum* and *F. oxysporum* (Mahlo et al. 2013). Moreover, strong antifungal activity of chloroform fraction of *Funtumia africana* (Benth.) Stapf was reported against *C. albicans* (Ramadwa et al. 2017).

Ethyl acetate fraction was further fractionated based on its strong antifungal activity. The results for antifungal activity are shown in Table 2. In the bioautography assay, only one compound was observed in the hexane fraction with an R_f

TABLE 1: Minimum inhibitory concentration (mg/mL) of solvent-solvent fractions of *Ximenia caffra* var. *natalensis*.

Plant species name	Solvent	MIC (mg/mL)
<i>X. caffra</i> var. <i>natalensis</i>	Hexane	0.31
	Chloroform	0.63
	Ethyl acetate	0.08
	Butanol	0.08
	Water	0.63

Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

Note: The results show the average of three replicates with a standard deviation of 0 in all cases.

MIC, minimum inhibitory concentration; *X. caffra*, *Ximenia caffra*.

value of 0.025 developed in BEA. The absence of single active compounds in these fractions suggests possible synergistic effects of compounds against *C. albicans*. It may be suggested that the separation of these compounds through solvent-solvent fractionation and further by TLC analysis resulted in their inactivity.

Bioassay-guided fraction led to the isolation of four compounds using silica gel (compounds 1 and 2) and Sephadex LH-20 column chromatography (compounds 3 and 4). Compounds 1 and 2 were purified using preparatory thin layer chromatography. Of all isolated compounds, only compound 1 was tested for antifungal activity. Compound 1 (Epigallocatechin gallate) was relatively active against *C. albicans* with a MIC value of 0.5 mg/ml.

Compound 1 was isolated as a red powder (20 mg). The mass spectrum showed the pseudo molecular peak at m/z 443.0941 $[M + H]^+$ and m/z 441.0821 $[M - H]^-$, consistent with the molecular formula $C_{22}H_{18}O_{10}$ (Figure 2). The 1H -NMR and ^{13}C -NMR data of Compound 1 are shown in Table 3. A search in the dictionary of natural products and chemical abstracts

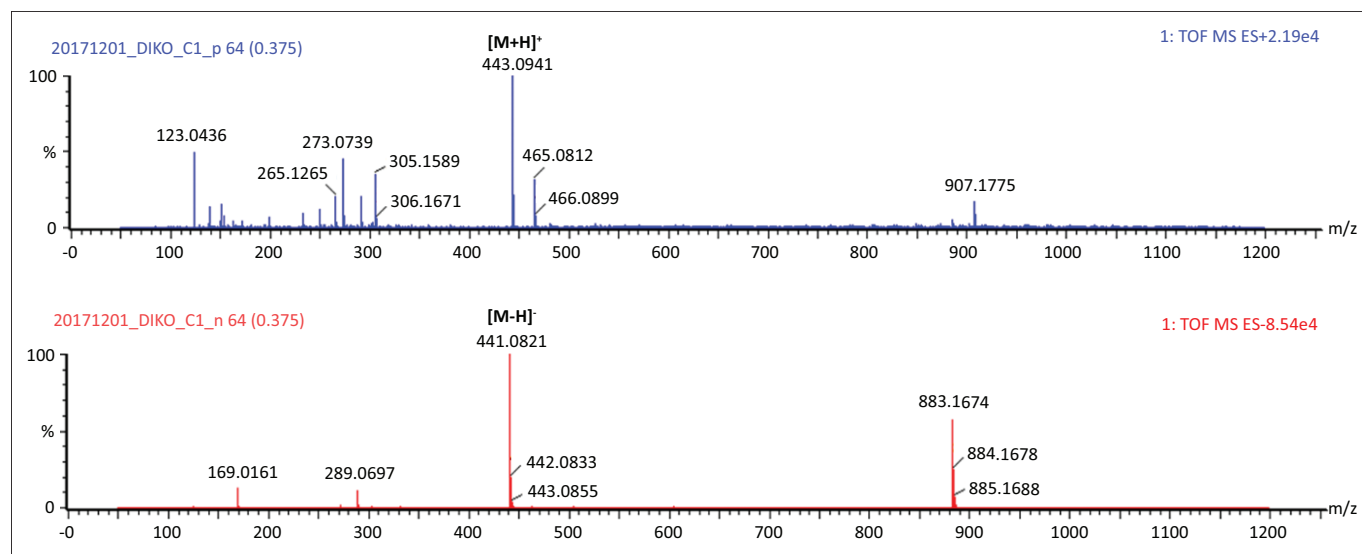
TABLE 2: Minimum inhibitory concentration values (mg/mL) of ethyl acetate sub-fractions obtained from silica gel column chromatography.

Fractions	MIC (mg/mL)
1	1.14
2	2.5
3	1.67
4	0.313
5	2.5
6	1.25
7	0.313
8	0.625
9	0.625
10	1.25

Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

Note: The results show the average of three replicates with a standard deviation of 0 in all cases.

MIC, minimum inhibitory concentration.



Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

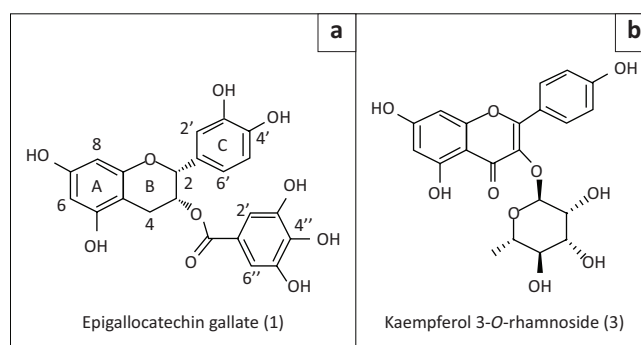
FIGURE 2: ESI/MS spectrum of Compound 1.

TABLE 3: $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of Compound (1) in $\text{DMSO-}d_6$.

Position	δ_c	C type	δ_H (mult.; J in [Hz])
2	77.0	CH	5.04 (s)
3	68.2	CH	5.35 (br s)
4	26.0	CH_2	2.95 (d, $^3J = 16.5$, 4.1 Hz) 2.69 (d, $^3J = 16.5$ Hz)
10	97.8	C_q	-
5	156.9	C_q	-
6	95.2	CH	5.84 (d, $^3J = 2.3$ Hz)
7	156.6	C_q	-
8	96.0	CH	5.90 (d, $^3J = 2.3$ Hz)
9	155.9	C_q	-
1'	131.0	C_q	-
2'	118.0	CH	6.68 (s)
3'	145.3	C_q	-
4'	144.5	C_q	-
5'	118.2	CH	6.77 (dd $^3J = 8.2$, 2.1 Hz)
6'	115.1	CH	6.87 (d $^3J = 2.1$ Hz)
1''	122.8	C_q	-
2'', 6''	108.5	CH	6.83 (s)
3'', 5''	145.0	C_q	-
4''	140.0	C_q	-
COO	166.5	C_q	-

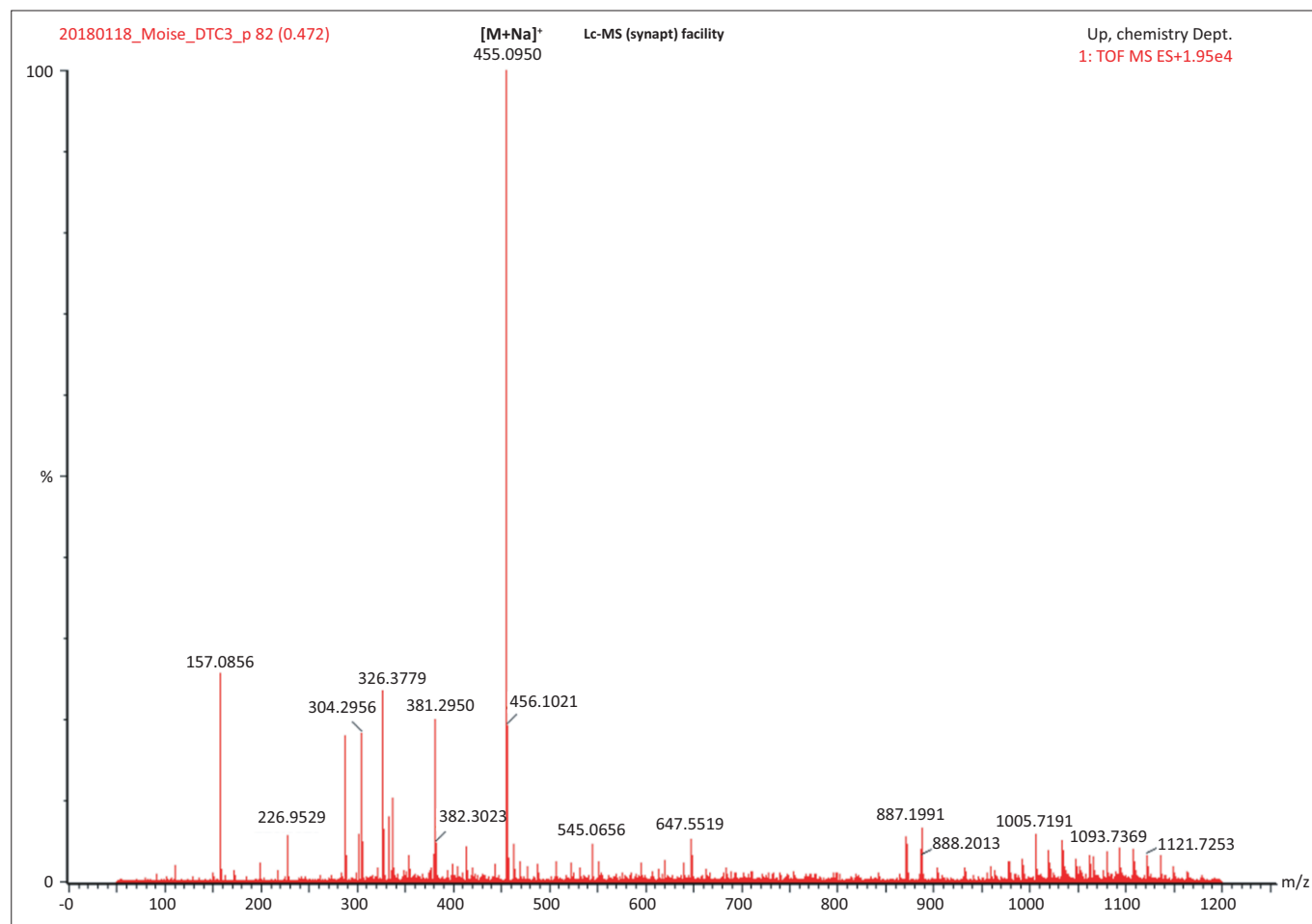
Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

services (Scifinder) using the spectroscopic information led to the structure of 1 as epigallocatechin gallate (EGCG) as shown in Figure 3, which was confirmed by comparing with the literature data (Zhang et al. 2016). The mass spectrum of Compound 3 revealed a pseudo molecular peak at m/z 455.0950 $[\text{M} + \text{Na}]^+$ and m/z 431.0996 $[\text{M} - \text{H}]^-$, giving it a molecular weight of 432 and the molecular formula $\text{C}_{21}\text{H}_{20}\text{O}_{10}$ by HRESIMS (Figure 4 and Figure 5). The compound was successfully identified as kaempferol 3-O-rhamnoside using



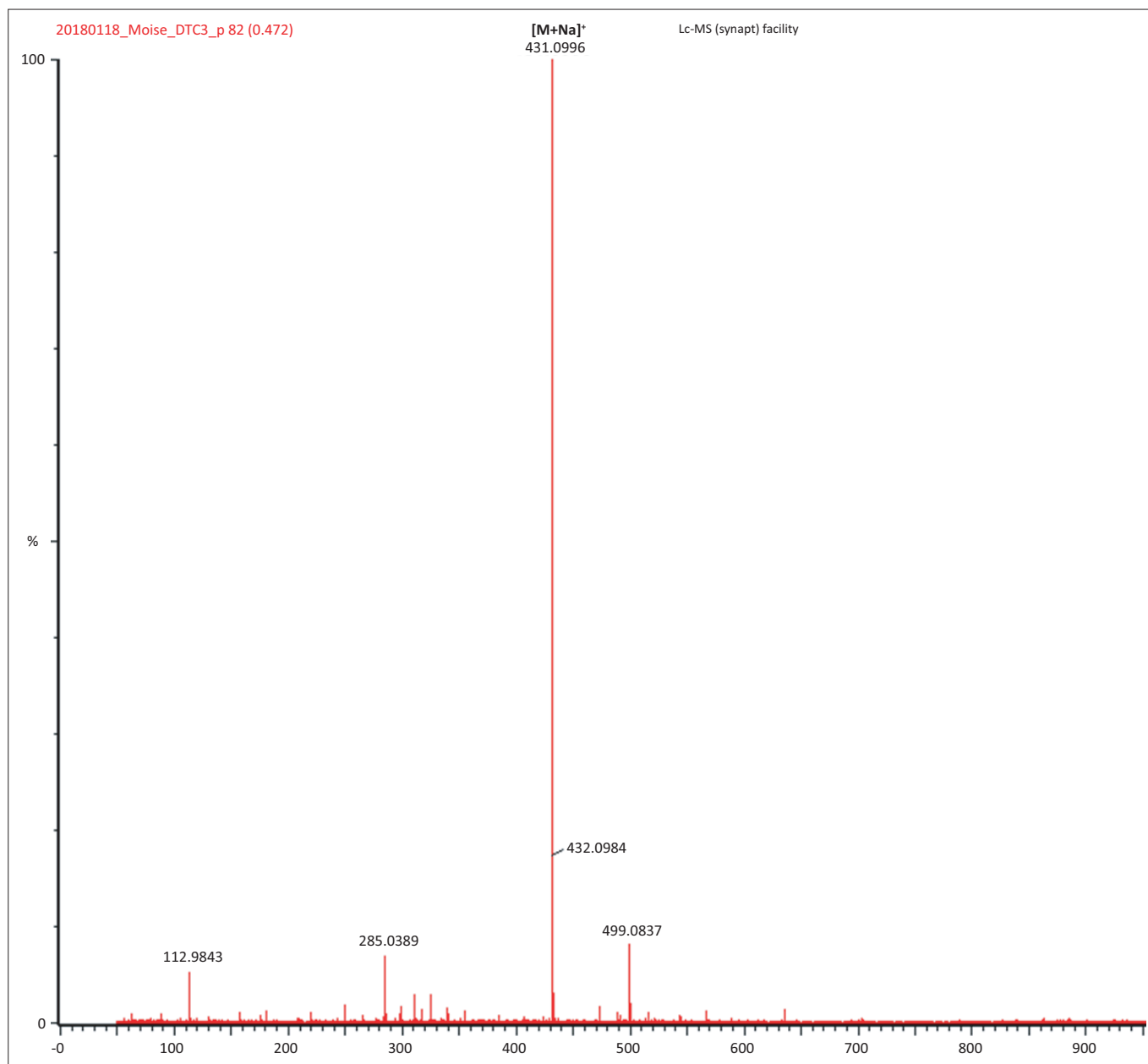
Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

FIGURE 3: Chemical structures of compounds 1 and 3 isolated from the ethyl acetate fraction of *Ximenia caffra* var. *natalensis*.



Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

FIGURE 4: Electrospray ionization mass spectrometry (ESIMS) for Compound 3 on positive mode.



Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69–80

FIGURE 5: Electrospray ionization mass spectrometry (ESIMS) for Compound 3 on negative mode.

the spectroscopic information. The spectral data is in agreement with the literature (Zhang et al. 2014). The chemical structures of kaempferol 3-O-rhamnoside are shown in Figure 3. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of Compound 3 are shown in Table 4. Compound 2 (7 mg) and Compound 4 (2 mg) were isolated as yellow powder.

Epigallocatechin gallate (EGCG) is a polyphenolic compound that is abundant in green tea extracts (*Camellia sinensis*). It constitutes 35%–52% of catechins in green tea with strong physiological activities including antimicrobial activity, antioxidant activity and induction of breast cancer apoptosis also reported the role of EGCG in lipid metabolism of the whole body as well as the cellular level (Zhang et al. 2016). The antiviral activity of EGCG against *Chikungunya* virus

was previously reported (Weber et al. 2015). Moreso, methanol root extracts of *X. americana* var. *caffra* are rich in catechins, including EGCG (Sobeh et al. 2017). EGCG isolated from the chloroform-ethyl acetate fraction of *Terminalia bellerica* fruits showed good antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Zhang et al. 2016).

Toxic effects of EGCG were determined against African green monkey kidney cells and this showed moderate toxicity at $\text{LC}_{50} = 32.32 \mu\text{g/ml}$. This validates the safety of EGCG as a potential compound for combating oral candidiasis.

Compound 3 (Kaempferol-3-O-rhamnoside) was previously isolated from ethyl acetate fraction of *Pometia pinnata*

TABLE 4: ¹H NMR and ¹³C NMR data of kaempferol-3-O-rhamnoside 2 (in DMSO-*d*₆).

Position	Compound 3	
	H	C
2	-	157.2
3	-	135.0
4	-	178.2
5	-	160.9
6	6.11 (s)	99.7
7	-	166.0
8	6.30 (s)	94.6
9	-	155.8
10	-	103.0
1'	-	121.5
2', 6'	7.72 (d, <i>J</i> = 8.2 Hz)	130.7
3', 5'	6.90 (d, <i>J</i> = 8.2 Hz)	115.9
4'	-	160.4
1''	5.30 (br s)	102.2
2''	3.98 (m)	70.5
3''	3.48 (ddd, <i>J</i> = 2.8, 8.7, 9.6 Hz)	70.8
4''	3.10 (dd, <i>J</i> = 3.5, 9.6 Hz)	71.5
5''	3.13 (dd, <i>J</i> = 6.3, 9.6 Hz)	71.0
6''	0.79 (d, <i>J</i> = 6.1 Hz)	17.9

Source: Tlaamela, D.M. & Mahlo, S.M., 2021, 'A survey of plant species used in traditional medicine for the treatment of various ailments in Aganang Local Municipality, Limpopo Province', *Indilinga African Journal of Indigenous Knowledge Systems* 20(1), 69-80

leaves (Utari et al., 2019). Furthermore, Kaempferol-3-O-rhamnoside isolated from ethyl acetate extract of *Schima wallichii* exhibited good anticancer activity against breast cancer cells (Diantini et al. 2012). The plasmodial activity of Kaempferol-3-O-rhamnoside against *Plasmodium falciparum* was reported (Barliana et al. 2013). The antioxidant activity of Kaempferol-3-O-rhamnoside isolated from *Tetraclinis articulata* was reported (Rached et al. 2018).

Conclusion

The results of our study demonstrate that butanol and ethyl acetate fractions of *X. caffra* var. *natalensis* had good antifungal activity against *C. albicans*. Bioassay-guided fractionation using silica gel and Sephadex LH-20 column chromatography of the ethyl acetate fraction led to the isolation and characterisation of two compounds. Structure elucidation of the isolated compounds *X. caffra* var. *natalensis* was determined using NMR and mass spectrometry. Compound 1 was successfully identified as epigallocatechin gallate (1) and compound 3 as kaempferol 3-O-rhamnoside. Furthermore, EGCG had moderate antifungal activity against *C. albicans*. This study indicates the potential of the ethyl acetate fraction to combat oral candidiasis. Bulk isolation of ethyl acetate fraction is recommended to further determine other active antifungal compounds present in the leaves of *X. caffra* var. *natalensis*.

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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, D.M.T., S.M., M.A. and L.M., designed the study, collected the data, and analysed and drafted the manuscript.

Ethical considerations

The study was approved by the University of Limpopo Research Committee (TREC/385/2017:PG).

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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, S.M.

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