J Herbmed Pharmacol. 2024; 13(3): 399-406.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2024.44959

Journal of Herbmed Pharmacology

Cytotoxicity and antimycobacterial activity of fractions and chemical constituents of *Lecaniodiscus cupanioides* Planch. Ex Benth

Olusesan Ojo[1*], Michael H.K. Kamdem[1], Edwin M. Mmutlane[2], Derek T. Ndinteh[1*]

[1]Centre for Natural Product Research (CNPR), Chemical Sciences Department, University of Johannesburg, Doornfontein, Johannesburg 2028, South Africa
[2]Research Centre for Synthesis and Catalysis, Department of Chemical Sciences, University of Johannesburg, Kingsway Campus, Auckland Park, P.O. Box 524, Johannesburg, South Africa

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 29 March 2023
Accepted: 9 August 2023

Keywords:
Mycobacterial infections
Lecaniodiscus cupanioides
Antimycobacterial activity
Cytotoxicity
Stigmasterol

ABSTRACT

Introduction: Mycobacterial infections remain a global problem that demands an urgent solution. *Lecaniodiscus cupanioides* Planch. ex Benth. is traditionally used as a medicinal plant to treat coughs, skin infections, cancers, sexual dysfunction, and malaria. This study focused on the antimycobacterial activity, cytotoxicity, and isolation of bioactive constituents of *L. cupanioides* chloroform leaves extract.

Methods: Antimycobacterial activity was assessed using the micro-dilution assay. Cytotoxic activity of the plant was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay. Column chromatography was used to purify the extract.

Results: Phytochemical investigation of *L. cupanioides* resulted in the isolation of three compounds, including eicosene (**1**), nonadecanol (**2**), and stigmasterol (**3**). The isolates **1**-**3** as well as the column fractions displayed antimycobacterial activity, with minimum inhibitory concentration (MIC) ranging from 0.50 to 1.0 mg/mL. The studied extract, column fractions, and isolated compounds exhibited no cytotoxicity against the human cervical cancer (HeLa) cell line.

Conclusion: The current results indicate that *L. cupanioides* has the potential to be employed in the formulation of herbal products or the development of new drugs against mycobacterial infections. However, more studies should be carried out using normal human cell lines to further unravel plant safety.

Implication for health policy/practice/research/medical education:
The phytochemical constituents of chloroform leaf extract of *Lecaniodiscus cupanioides* were identified, isolated, and discovered to have antimycobacterial properties, especially for managing infections, such as skin or soft-tissue, pneumonia, bacteremia, and bone diseases clinically caused by *Mycobacterium smegmatis*.

Please cite this paper as: Ojo O, Kamdem MHK, Mmutlane ED, Ndinteh DT. Cytotoxicity and antimycobacterial activity of fractions and chemical constituents of Lecaniodiscus cupanioides Planch. Ex Benth. J Herbmed Pharmacol. 2024;13(3):399-406. doi: 10.34172/jhp.2024.44959.

Introduction

Mycobacterial infections remain a global concern, with an estimation of more than 9.4 million cases yearly (1). In addition to pulmonary tuberculosis, which is recognized as the most prevalent form of mycobacterial infections, other forms of extra-pulmonary mycobacterial infections are on the increase annually (2). *Mycobacterium smegmatis* is a fast-growing bacterium under the family Mycobacteriaceae, which also encompasses the pathogenic strains, including *Mycobacterium tuberculosis, Mycobacterium bovis,* and *Mycobacterium fortuitum* (3-5). Although infections caused by *M. smegmatis* are rare unlike *M. tuberculosis*, they are nevertheless clinically important pathogens. Several cases of infections, such as skin or soft tissue, pneumonia, bacteremia, and bone diseases clinically caused by *M. smegmatis* have been reported in immunocompromised individuals (6-10). Moreover, *M. smegmatis* has been shown to be resistant to

***Corresponding authors**: Derek T. Ndinteh, Email: dndinteh@uj.ac.za; Olusesan Ojo, Email: dndinteh@uj.ac.za

multiple clinical drugs, including rifampicin used currently for the treatment of the deadly disease, tuberculosis (11,12). Besides, *M. smegmatis* has gained an increasing acceptability as a target in prior anti-tuberculosis drug discovery where access to biosafety level 3 (BSL-3) facility is unavailable to select the best drug candidates for further screening against *M. tuberculosis* (13-18). Hence, the discovery and designing of new antimycobacterial agents is an urgent business for the treatment of ever-increasing mycobacterial infections, including tuberculosis.

For decades, herbal therapy has been traditionally employed in different local communities across the globe for the treatment of diseases. This is partly due to the belief that herbal products or medicines have unusual preventive and curative properties, and that they are widely available and cheaper than the conventional drugs (19). The plant *Lecaniodiscus cupanioides* Planch. Ex Benth. (Sapindaceae) is one of the two accepted species of the genus *Lecaniodiscus* (http://www.theplantlist.org/); it is often found in deciduous and non-deciduous rain forests, especially in Africa and Asia. It is a small shrub plant about 6-12 m high or more (20). It is ethnobotanically documented that the decoction of the plant's root is used to cure the problem of sexual dysfunction in sub-Saharan Africa (21). It is also used to treat tuberculosis, coughs, skin infections, jaundice, cancers, and malaria (22). *L. cupanioides* has been scientifically reported to act as an anti-depressant (23), aphrodisiac (24), anti-fungal (25), antimicrobial, anti-tumour (26) and anti-malarial plant (27). Chemical constituents isolated from *L. cupanioides,* as reported in the literature, are triterpenoid saponins (28,29) and sesquiterpene glycosides (29). The isolated compounds, such as triterpenoid saponins are reported to show anticancer and antifungal activities (30,31). Meanwhile, it has been stressed that many of the extracts or compounds with known antifungal activity are also known to inhibit *Mycobacteria* (32,33). Therefore, *L. cupanioides*, having known reported antifungal activity, was screened against the strain of *Mycobacteria*. To the best of our knowledge, there is no literature available on the antimycobacterial activity of *L. cupanioides*. The present work, therefore, evaluated the antimycobacterial activity and the cytotoxic effect of the column fractions and the isolated constituents from *L. cupanioides* leaves.

Materials and Methods

General experimental procedures

Nuclear magnetic resonance (NMR) spectrometer (Brucker Avance III version) was used to record the ^{1}H and ^{13}C NMR spectra of the isolated compounds at 500 and 125 MHz, respectively. Tetramethylsilane (TMS) was used as the internal standard. Deuterated chloroform (d-$CHCl_3$) and dimethyl sulphoxide (d-DMSO) were used as solvents for running the NMR analysis. Mass spectra (MS) were recorded on a Brucker Compact mass spectrometer (University of the Witwatersrand, South Africa). Infra-Red (IR) analysis was performed using Perkin Elmer FTIR 600 series. Column chromatography was performed using column (3 cm × 55 cm) with silica gel (mesh size; 60–120). Thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} plates (Macherey-Nagel GmbH & Co. KG) with layer thickness of 0.20 mm. The TLC plates were treated with *p*-anisaldehyde-sulphuric acid reagent and visualized under ultraviolet (UV) light at 254 and 365 nm. The melting points were carried out using Stuart melting point SMP30 apparatus. Chemicals used in the experiment were purchased from Sigma-Aldrich, South Africa.

Plant sample collection and extract preparation

The *L. cupanioides* fresh leaves were collected at the University of Ibadan, Ibadan, Nigeria (Geographical location: 7° 23' 28N 3° 54' 60E) in September, 2019. It was authenticated by Mr. D. P. O. Esimekhuai from the Botany Department, University of Ibadan, Nigeria. The voucher specimen of the plant was subsequently deposited at the Department of herbarium, with the voucher specimen number UIH-22898. The collected leaves were thoroughly washed with cold water to remove unwanted materials, such as dirt, and thereafter air-dried in a shaded open place for 2 weeks. The air-dried leaves were powdered using an industrial grinder and stored in a sealed polythene bag until further use. The milled leaves sample was extracted by soaking 250 g of the sample in chloroform (1 L) at room temperature for 24 hours, and successively repeated for 5 times. The Whatman filter paper No. 1 was utilized to filter the extracting chloroform from the powdered material and concentrated using a rotary vacuum evaporator (Buchi rotary evaporator) at 40 °C. The obtained dark-brown crude extract (15 g) was kept until further studies.

Chromatography separation of the crude extract

The extract (8 g) was subjected to the chromatography separation using a column containing 200 g of silica gel. Gradient elution was used viz - hexane (100, *v*), hexane-ethyl acetate (98:2, *v/v*), hexane-ethyl acetate (96:4, *v/v*), hexane-ethyl acetate (90:10, *v/v*), hexane-ethyl acetate (85:15, *v/v*), hexane-ethyl acetate (80:20, *v/v*), hexane-ethyl acetate (75:25, *v/v*), hexane-ethyl acetate (70:30, *v/v*), hexane-ethyl acetate (60:40, *v/v*), hexane-ethyl acetate (20:80, *v/v*), ethyl acetate (100, *v*), ethyl acetate-methanol (95:5, *v/v*), and ethyl acetate-methanol (90:10, *v/v*) to obtain 159 fractions (100 mL each). The fractions were pooled together into seven sub-fractions coded LCC 1 (12.1 mg), LCC 2 (23.0 mg), LCC 3 (24.0 mg), LCC 4 (28.0 mg), LCC 5 (44.0 mg), LCC 6 (32.5 mg), and LCC 7 (890.0 mg) based on their TLC analysis. Sub-fraction LCC 1 gave one spot on TLC (solvent system: 100% hexane), and compound **1**, a white amorphous solid, was obtained.

The further purification of sub-fractions LCC 2, LCC 3, LCC 5, and LCC 7 (Figure 1) failed to yield pure isolates, while sub-fraction LCC 4 eluted with hexane-ethyl acetate (85:15, *v/v*), on repeated washing in *n*-hexane and recrystallization in dichloromethane-methanol (DCM-MEOH), yielded white needle-like crystal compound **2** (24.2 mg). Compound **3** (22.4 mg), eluted with hexane-ethyl acetate (80:20 to 75:25, *v/v*), was obtained as a white crystal solid from sub-fraction LCC 6 upon repeated washing in *n*-hexane and recrystallization in methanol-ethyl acetate (MEOH-EtOAc). The structures of the compounds **1-3** were unambiguously elucidated based on their obtained physical and spectroscopic data as well as comparison with those published in the literature.

Compound 1 (1-eicosene): White solid; ESI-MS: $[M+H]^+$ = 281.2407; MF: $C_{20}H_{40}$; soluble in $CHCl_3$; ^{13}C NMR (125 MHz, δ ppm, d-$CHCl_3$ = 77.23): 114.30 (CH_2, C_1), 139.44 (CH=, C_2), 37.39 (CH_2, C_3), 34.08 (CH_2, C_4), 29.24 (CH_2, C_5), 29.43 (CH_2, C_6), 29.64 (CH_2, C_7), 30.32 (CH_2, C_8), 29.98 (CH_2, $C_{9\text{-}16}$), 27.37 (CH_2, C_{17}), 32.20 (CH_2, C_{18}), 22.96 (CH_2, C_{19}), 14.34 (CH_3, C_{20}); ^1H-NMR (500 MHz, δ ppm, $CHCl_3$-d = 7.24): 4.92 (2H, dd, *J* = 10.2 Hz, H_1), 5.78 (1H, m, *J* = 10.5 Hz, H_2), 2.01 (2H, m, *J* = 8.0 Hz, H_3), 1.24 (2H, m, $H_{4\text{-}19}$), 0.88 (3H, t, H_{20}). These data are in accordance with those reported for 1-eicosene in the literature (34).

Compound 2 (**1-nonadecanol**): White needle-like crystal; ESI-MS: $[M+H]^+$ = 285.1619; MF: $C_{19}H_{39}OH$; soluble in $CHCl_3$-DMSO mixture; melting point = 60 – 62 °C (uncorrected); R_f = 0.54 on pre-coated silica gel plate 60 F_{254} (Hex/EtOAc, 8:2); ^1H-NMR (500 MHz, δ ppm, $CHCl_3$-d = 7.24): 0.81 (3H, t, *J* = 6.7 Hz, H_{19}), 1.19 (2H, m, $H_{2\text{-}18}$), 2.17 (1H, br, OH), 3.55 (2H, t, *J* = 7.3 Hz, H_1); ^{13}C NMR (125 MHz, δ ppm, d-$CHCl_3$ = 77.23, DMSO-d = 40.17): 62.90 (CH_2OH, C_1), 32.92 (CH_2, C_2), 25.60 (CH_2, C_3), 25.54 (CH_2, C_4), 29.61 (CH_2, $C_{5\text{-}15}$), 29.42 (CH_2, C_{16}), 32.00 (CH_2, C_{17}), 22.60 (CH_2, C_{18}), 14.16 (CH_3, C_{19}). These data are in accordance with those reported in the literature (35).

Compound 3 (stigmasterol): White crystal solid; ESI-MS: $[M+H]^+$ = 413.3627; MF = $C_{29}H_{48}O$; m. pt. = 135.2 – 136.8 °C; soluble in $CHCl_3$-d; ^1H-NMR (500 MHz, δ ppm, $CHCl_3$-d = 7.24): 3.50 (1H, tdd, *J* = 6.3 Hz, H_3), 5.33 (1H, t, H_6), 5.12 (1H, m, *J* = 8.6 Hz, H_{22}), 5.00 (1H, m, *J* = 8.5 Hz, H_{23}), 1.23 (3H, s), 1.05 (3H, s), 0.99 (3H, s), 0.82 (3H, s), 0.79 (3H, s), 0.67 (3H, s); ^{13}C NMR (125 MHz, δ ppm, d-$CHCl_3$ = 77.23): 37.49 (CH_2, C_1), 39.92 (CH_2, C_2), 72.02 (CH, C_3), 42.55 (CH_2, C_4), 140.99 (QC=, C_5), 121.90 (CH=, C_6), 29.10 (CH_2, C_7), 32.09 (CH, C_8), 51.46 (CH, C_9), 36.74 (QC, C_{10}), 21.30 (CH_2, C_{11}), 31.91 (CH_2, C_{12}), 42.45 (QC, C_{13}),50.42 (CH, C_{14}), 24.58 (CH_2, C_{15}), 25.60 (CH_2, C_{16}), 56.22 (CH, C_{17}), 12.26 (CH_3, C_{18}), 21.42 (CH_3, C_{19}), 40.66 (CH, C_{20}), 19.60 (CH_3, C_{21}), 129.53 (CH, C_{22}), 138.49 (CH, C_{23}), 57.10 (CH, C_{24}), 32.14 (CH, C_{25}), 21.26 (CH_3, C_{26}), 12.43 (CH_3, C_{27}), 32.12 (CH_2, C_{28}), 19.20 (CH_3, C_{29}). These data are in tandem with those reported by Kaigongi et al (36).

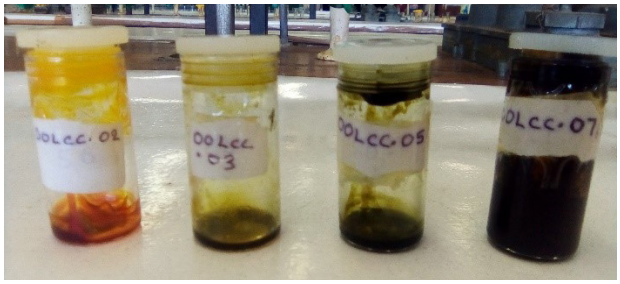

Figure 1. Samples of column fractions (LCC 2, LCC 3, LCC 5, and LCC 7) obtained from *Lecaniodiscus cupanioides* leaves chloroform extract.

Qualitative phytochemical analysis

In order to determine the nature of chemical compositions of the unresolved fractions LCC 2, LCC 3, LCC 5, and LCC 7 obtained from the column (Figure 1), qualitative phytochemical analysis was carried out. The established standard procedures for phenols and terpenoids as described by Harborne (37), for a steroid the method of Nath et al (38), for flavonoids and tannins the method of Bohm and Koupai-Abyazani's (39), and for saponins the method of El Aziz et al (40) were used (Table 1).

Antimycobacterial testing

Microbial culture

Mycobacterium smegmatis (MC 2155) was sourced from the Food and Biotechnology Department, University of Johannesburg, South Africa. Prior to its use, the *M. smegmatis* strain was cultured on Middlebrook 7H11 agar (Becton Dickinson, Sparks, MD) under hygienic conditions, supplemented with 2% (v/v) glycerol and 10% (v/v) oleic acid, albumin, dextrose and catalase, and allowed to grow for 24 hours.

Table 1. Phytochemical constituents of the column fractions obtained from *Lecaniodiscus cupanioides* leaves extract

Column fractions	Saponins	Flavonoids	Terpenoids	Tannins	Phenols	Steroids
LCC 2	-	+++	-	-	-	-
LCC 3	-	+++	+++	-	-	-
LCC 5	-	+++	+++	-	-	-
LCC 7	-	-	-	-	-	-

+++: very abundant; -: not detected; LCC 2, 3, 5, and 7: Fractions from chloroform leaves extract of *Lecaniodiscus cupanioides*.

Table 2. Minimum inhibitory concentrations (MICs) of isolated compounds and column fractions obtained from *Lecaniodiscus cupanioides* leaves extract

Sample	LC C 1	LCC 2	LCC 3	LCC 4	LCC 5	LCC 6	LCC 7	SM
MIC (mg/mL)	0.50	0.50	0.50	0.50	0.50	1.00	0.50	0.004

LCC 1: compound 1 (1-eicosene); LCC 2: fraction; LCC 3: fraction; LCC 4: compound 2 (1-nonadecanol); LCC 5: fraction; LCC 6: compound 3 (stigmasterol); LCC 7: fraction; SM: streptomycin

Minimum inhibitory concentrations (MICs) determination

Both the column fractions and the isolated pure compounds were screened for antimycobacterial activity against *M. smegmatis*. The MICs of the test samples were determined using broth microdilution techniques as described by Wardani et al (41), with little modifications. Briefly, each test sample was dissolved in DMSO, and the final concentration of each test sample was diluted serially (two-folds dilution) to afford six working concentrations between 1.0 and 0.031 mg/mL. 100 µL of Middlebrook 7H9 broth was dispensed into each well of the microtitre plate mixed with 100 µL of each test sample concentration under aseptic conditions. Each microtitre plate was tightly sealed with aluminium foils and incubated at 37 °C for 24 hours. Resazurin solution (40 µL of 200 µg/mL) was added after the completion of incubation period, and the plates were re-incubated for 2 hours at 37 °C to confirm viable bacterial cells as the colour changed from blue to pink, and remained blue in dead bacterial cells. The lowest concentration that prevented the change in colour from blue to pink was taken as MIC (42). Streptomycin (SM), one of the first-lines for TB chemotherapy, was employed as positive control to compare the MICs of the test compounds and column fractions (43) (Table 2).

Cytotoxicity assay

The *in vitro* cytotoxicity studies of the extract, the fractions and the compounds **1**-**3** were evaluated using MTT-based assay against human cervical cancer cell line (HeLa cells) as previously described by Parthasarathy et al (44) with little modifications. In this assay (a measure of mitochondrial function), the MTT (a yellow tetrazole) is enzymatically reduced to formazan (purple) by the action of nicotinamide adenine dinucleotide (NADH) in metabolically active cells (45). Briefly, fixed concentration (50 µg/mL) of tested samples was prepared in 100% analytical-graded dimethyl sulphoxide (DMSO) and incubated with HeLa cells in 96-well plate (1×10^4 cells/mL) cultured and maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% (v/v) fetal bovine serum (FBS) at a temperature of 37 °C in an environment of 5% carbon dioxide for 1 day. The control cells were supplemented with 100 µL with pure DMSO. The number of HeLa cells that survived the tested samples exposure were evaluated by using the resazurin-based reagent and reading resorufin fluorescence in a multiwell micro-plate reader (VERSAmax, USA) at 595 nm. All experiments were performed in duplicate, and a mean standard deviation (SD) was calculated for each. The data represented as the percentage (%) cell viability based on the fluorescence reading in treated wells against untreated wells (Table 3).

Results

Structure elucidation of compounds **1**-**3**

The chloroform leaves extract of *L. cupanioides* was purified with column chromatography over silica gel, leading to the isolation of three known compounds namely 1-eicosene (**1**), 1-nonadecanol (**2**), and stigmasterol (**3**) (Figure 2). The compounds **1**-**3** were structurally elucidated by the analysis of their NMR (1D- and 2D-NMR) experiments, FTIR, EIS-MS, and by comparison with data published in scientific literature. The compounds **1**-**3** were isolated for the first time from *L. cupanioides* to the best of our knowledge.

Compound **1** was obtained as a white solid. ESI-MS (positive mode) exhibited molecular ion peak at *m/z* 281.2407, giving molecular formula of $C_{20}H_{40}$. The ^{1}H-NMR spectrum of compound **1** revealed signal at 4.92 ppm (2H, *J* = 10.2 Hz) assigned to the vinylic geminal protons at C_1. The signal at 5.78 ppm (1H, *J* = 10.5 Hz) was assigned to vinylic proton at C_2. The signal at 2.01 ppm (2H, *J* = 8.0 Hz) was assigned to allylic protons at C_3. The signal at 1.24 ppm was assigned to methylene protons (-CH_2-) while the signal at 0.88 ppm was assigned to methyl protons at C_{20}. The ^{13}C-NMR and DEPT-135 NMR experiment of **1** showed the presence of one methyl carbon, eighteen methylene carbons, and one methine carbon, suggesting that compound **1** should be aliphatic strain-chain alkene. ^{13}C-NMR spectrum showed the presence of signals at 114.30 and 139.44 ppm and were both assigned to carbon atoms at C_1 and C_2, respectively.

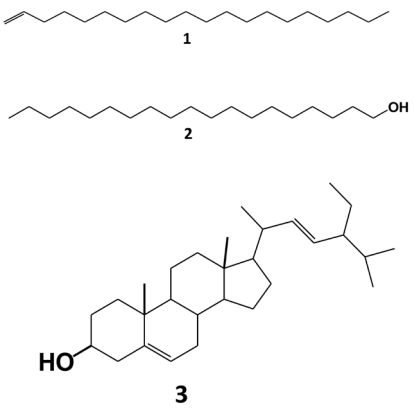

Figure 2. Compounds **1** (1-eicosene), **2** (1-nonadecanol), and **3** (stigmasterol) isolated from *Lecaniodiscus cupanioides* leaves.

Table 3. Cytotoxicity profile of *Lecaniodiscus cupanioides* crude extract, column fractions, and isolated pure compounds

Samples tested at 50 µg/mL	IC_{50} (µg/mL)
LCC 1	79.08
LCC 2	75.85
LCC 3	54.78
LCC 4	71.01
LCC 5	61.38
LCC 6	75.99
LCC 7	53.10
Crude extract	61.70

LCC 1: compound 1 (1-eicosene); LCC 2: fraction; LCC 3: fraction; LCC 4: compound 2 (1-nonadecanol); LCC 5: fraction; LCC 6: compound 3 (stigmasterol); LCC 7: fraction; IC50: Half-maximal inhibitory concentration.

The signal at 37.39 ppm was assigned to carbon atom at C_3. The signals at 34.08, 29.24, 29.43, 29.64, 30.32, 29.98, 27.37, 32.20, and 22.96 ppm were assigned to other 16 methylene carbon atoms. The methyl carbon signal at 14.34 ppm was assigned to carbon at C_{20}. FTIR showed the presence of C=C bond with absorption frequency at 1632.0 cm^{-1}. The absorption bands at 2913.9 and 2841.0 cm^{-1} signified the aliphatic -CH stretching vibration. In general, based on the spectroscopic data (IR, NMR and MS) and comparison with data in the scientific literature, the structure of the isolated compound **1** was determined to be 1-eicosene.

Compound **2** was obtained as a white needle-like crystal. Its ESI-MS (positive mode) revealed molecular ion peak at *m/z* 285.1619, giving molecular formula of $C_{19}H_{39}OH$. ^{13}C-NMR and DEPT-135 experiments showed nine signals, of which signals at 14.18 ppm and 62.96 ppm were due to the presence of a -CH_3 and -CH_2OH, respectively. The remaining signals resonating at 22.76 – 32.92 ppm were assigned to other aliphatic -CH_2 groups, suggesting that compound 2 should be aliphatic straight-chain primary alcohol. The ^{1}H-NMR spectrum of compound **2** revealed a downfield signal at 3.55 ppm (2H, t, J = 7.3 Hz), which is unambiguously assigned to -CH_2OH group. This feature was confirmed by FTIR spectrum with a broad absorption band of alcoholic -OH stretching at 3450 cm^{-1} and -C-O_{str} band of primary alcohol at 1076 cm^{-1}. The signal at 0.81 ppm (3H, t, J = 6.7 Hz) was assigned to the terminal methyl protons. An upfield broad signal at 1.19 ppm (34H, m) was assigned to H_2 to H_{18} and was confirmed by C-H bending absorption band of repeated aliphatic methylene groups at 740 cm^{-1}. Based on the above data together with those in the literature, compound **2** was identified as 1-nonadecanol.

Compound **3** was isolated as a white crystal solid and showed a positive test with Liebermann-Burchard reagent confirming the presence of steroidal nucleus (38). It gave a quasi-molecular ion peak at *m/z* 413.3627, corresponding to the molecular formula of $C_{29}H_{48}O$. ^{13}C-NMR and DEPT-135 experiments of compound **3** showed 29 carbons comprising six methyl (CH_3) groups, nine methylene (CH_2) carbons, eleven methine (CH) carbons, and three quaternary (C) carbons. The downfield signals at 140.99, 121.90, 138.49, and 129.53 ppm were assigned to C_5, C_6, C_{22}, and C_{23}, respectively, indicating the presence of unsaturation. The signal at 72.02 ppm (C_3) is due to the presence of β-hydroxyl group (-OH) at C_3. The angular carbon atom resonances at C_{19} and C_{18} were recognized by the signals at 21.42 and 12.26 ppm, respectively. The ^{1}H-NMR spectrum showed three olefinic proton signals at 5.00 (H_{23}), 5.12 (H_{22}), and 5.33 (H_6) ppm, a proton on an oxygenated carbon at 3.50 ppm (H_3) together with a cluster of signals between 2.26 – 0.65 ppm, suggesting a steroidal nucleus. FTIR showed the characteristic -OH stretching vibration at 3362.0 cm^{-1}. The bands at 2913.9 and 2848.0 cm^{-1} are the characteristic aliphatic C-H stretching vibrations while the band at 1627.9 cm^{-1} is absorption for C=C bond vibration. The absorption frequency at 1027.1 cm^{-1} is due to cycloalkane. The band at 757.4 cm^{-1} signifies the C-H out-of-plane vibration.

Qualitative phytochemical profile

The phytochemical compositions of the unresolved column fractions LCC 2, LCC 3, LCC 5, and LCC 7 are depicted in Table 1, with the identified phytochemical constituents being the flavonoids and terpenoids. These secondary metabolites might be linked to the plant usage in combating infections and diseases. However, none of the tested secondary metabolites was present in LCC 7.

Minimum inhibitory concentrations determination

The results of the antimycobacterial activity of *L. cupanioides* column fractions and the three isolates are shown in Table 2. The tested samples exhibited moderate antimycobacterial activity, with MICs ranging from 0.50 to 1.0 mg/mL. However, the highest MIC was observed with pure isolated compound **3** (stigmasterol).

Cytotoxicity of the studied samples

Table 3 and Figure 3 show the results of the cytotoxic activity of the studied samples against HeLa cells. All the tested compounds, the column fractions, and the extract exhibited IC_{50} values below 80 µg/mL. The column fractions LCC 3 and LCC 7 gave the best activity of all the tested samples. However, the highest value was observed for LCC 1 (1-eicosene) (Table 3).

Discussion

Thus, this study investigated the antimycobacterial efficacy of the column fractions and the isolated compounds **1**-**3** from the chloroform leaves extract against *M. smegmatis*. To establish whether the extract, the column fractions, and the isolated compounds contributed to the cytotoxicity of the plant, they were screened against the human cervical

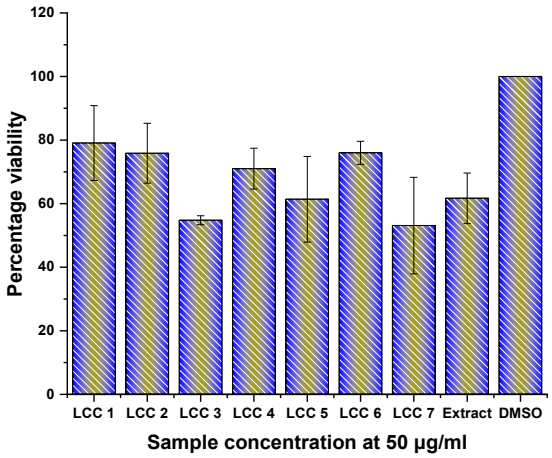

Figure 3. Cytotoxic activity of the test samples from *Lecaniodiscus cupanioides* leaves at single concentration (50 µg/mL). Dimethyl sulphoxide (DMSO) was used as a negative control. LCC 1: compound **1** (1-eicosene); LCC 2: fraction; LCC 3: fraction; LCC 4: compound **2** (1-nonadecanol); LCC 5: fraction; LCC 6: compound **3** (stigmasterol); LCC 7: fraction.

cancer cell line (HeLa) using the MTT assay.

The column fractions and the compounds **1**-**3** showed moderate growth inhibition of *M. smegmatis* (Table 2). The phytochemical screening of the fractions showed the presence of flavonoids and terpenoids, with the exception of column fraction LCC 7 (Table 1). These secondary metabolites, flavonoids, and terpenoids represent two of the most diverse and structural groups of phytochemicals in plants (46,47). These secondary metabolites have been linked with several pharmacological activities, including antimycobacterial activity, which supports the findings in this study (48-51). In addition, this study supports the previous findings on the antimycobacterial activity of compound **3** (stigmasterol) (52). Fomogne-Fodjo et al (53) reported that the isolated compound **3** displayed appreciable inhibition of both *M. smegmatis* and *Mycobacterium aurum* with a MIC of 15 µg/mL. There is no information on the antimycobacterial activity of compound **1** (1-eicosene) and compound **2** (nonadecanol) in the available literature as shown in this study. However, compound **2** has been recently profiled in a plant extract that exhibited the inhibition of *M. smegmatis* (54). The *in vitro* cytotoxicity of the extract, fractions, and compounds **1**-**3** showed non-cytotoxicity at the single concentration (Figure 3) tested against HeLa cell line in this study according to the American National Cancer Institute (NCI) benchmark. Based on NCI protocol, the limit for the cytotoxicity of a plant extract is an IC_{50} value ≤ 20 µg/mL, while pure compounds are considered to be cytotoxic at an IC_{50} value ≤ 4 µg/mL (55). In contrast to our study, Ogbole et al (56) reported the cytotoxic effect of *L. cupanioides* methanol leaves extract against Rhabdomyosarcoma (RD) cell line (CC_{50} = 17.23 ± 1.98 µg/mL). The non-cytotoxicity of *L. cupanioides* in the present study might be attributed to the minor compounds of the chloroform extract, and its phytochemicals. However, further studies using normal human cell lines would be valuable to understand the role of these extracts in the cytotoxicity of the plant for safety use. The present study could be improved by using two or more pathogenic strains of *M. tuberculosis* in a well-equipped biosafety level 3 (BSL-3) facility and studying the mechanism of actions of both the pure compounds and the chromatographic fractions.

Conclusion

Lecaniodiscus cupanioides is an underutilized medicinal plant traditionally used to treat coughs, skin infections, jaundice, cancers, sexual dysfunction, and malaria. The phytochemical investigation of *L. cupanioides* resulted in the isolation of three known compounds, including eicosene (**1**), nonadecanol (**2**), and stigmasterol (**3**). To our knowledge, compounds **1**-**3** were isolated from *L. cupanioides* for the first time. The isolated compounds and the column fractions showed the inhibition of *M. smegmatis* (MICs = 0.50 - 1.0 mg/mL). Also, the studied extract, the fractions, and the isolated compounds showed no-cytotoxicity against HeLa cell line. The current results indicated that *L. cupanioides* has the potential to be employed in the development of herbal drugs or the formulation of herbal products against mycobacterial infections. However, further studies should be carried out using normal human cell lines to address the plant safety.

Authors' contribution

Conceptualization: Olusesan Ojo and Derek T. Ndinteh.
Data curation: Olusesan Ojo and Derek T. Ndinteh.
Formal analysis: Olusesan Ojo, Derek T. Ndinteh and Michael H.K. Kamdem.
Funding acquisition: Olusesan Ojo and Derek T. Ndinteh.
Methodology: Olusesan Ojo and Derek T. Ndinteh
Project administration: Olusesan Ojo and Derek T. Ndinteh.
Supervision: Derek T. Ndinteh and Edwin M. Mmutlane.
Writing–original draft: Olusesan Ojo.
Writing–review & editing: Derek T. Ndinteh and Edwin M. Mmutlane.

Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues regarding data acquisition, analysis, authorship, review, duplication of work and plagiarism have been carefully observed by the authors. This study does not involve humans or animals. Therefore, ethical consideration with respect to human and animal use is not required.

Funding

This work was supported financially by the National

Research Foundation, South Africa (Ref: Grant Number 139057).

References

1. Zeller S, Ferneini EM. Tuberculosis and mycobacterial infections of the head and neck. In: Head, Neck, and Orofacial Infections. St. Louis: Elsevier; 2016. p. 416-21. doi: 10.1016/b978-0-323-28945-0.00031-4.
2. Shih DC, Cassidy PM, Perkins KM, Crist MB, Cieslak PR, Leman RL. Extrapulmonary nontuberculous mycobacterial disease surveillance - Oregon, 2014-2016. MMWR Morb Mortal Wkly Rep. 2018;67(31):854-7. doi: 10.15585/mmwr.mm6731a3.
3. Zimhony O, Vilchèze C, Jacobs WR Jr. Characterization of *Mycobacterium smegmatis* expressing the *Mycobacterium tuberculosis* fatty acid synthase I (fas1) gene. J Bacteriol. 2004;186(13):4051-5. doi: 10.1128/jb.186.13.4051-4055.2004.
4. Torres-Gonzalez P, Cervera-Hernandez ME, Martinez-Gamboa A, Garcia-Garcia L, Cruz-Hervert LP, Bobadilla-Del Valle M, et al. Human tuberculosis caused by *Mycobacterium bovis*: a retrospective comparison with *Mycobacterium tuberculosis* in a Mexican tertiary care centre, 2000-2015. BMC Infect Dis. 2016;16(1):657. doi: 10.1186/s12879-016-2001-5.
5. Wong KP, Tang ZH, Tan GM. *Mycobacterium fortuitum* and *Mycobacterium abscessus* infections in the foot and ankle in two immunocompetent patients. Biomedicine (Taipei). 2020;10(4):52-6. doi: 10.37796/2211-8039.1021.
6. Best CA, Best TJ. *Mycobacterium smegmatis* infection of the hand. Hand. 2009;4(2):165-6. doi: 10.1007/s11552-008-9147-6.
7. Butt S, Tirmizi A. *Mycobacterium smegmatis* bacteremia in an immunocompetent host. IDCases. 2019;15:e00523. doi: 10.1016/j.idcr.2019.e00523.
8. Ergan B, Coplu L, Alp A, Artvinli M. *Mycobacterium smegmatis* pneumonia. Respirology. 2004;9(2):283-5. doi: 10.1111/j.1440-1843.2004.00570.x.
9. Pierre-Audigier C, Jouanguy E, Lamhamedi S, Altare F, Rauzier J, Vincent V, et al. Fatal disseminated *Mycobacterium smegmatis* infection in a child with inherited interferon gamma receptor deficiency. Clin Infect Dis. 1997;24(5):982-4. doi: 10.1093/clinids/24.5.982.
10. Wallace RJ Jr, Nash DR, Tsukamura M, Blacklock ZM, Silcox VA. Human disease due to *Mycobacterium smegmatis*. J Infect Dis. 1988;158(1):52-9. doi: 10.1093/infdis/158.1.52.
11. Arthur PK, Amarh V, Cramer P, Arkaifie GB, Blessie EJS, Fuseini MS, et al. Characterization of two new multidrug-resistant strains of *Mycobacterium smegmatis*: tools for routine in vitro screening of novel anti-mycobacterial agents. Antibiotics (Basel). 2019;8(1):4. doi: 10.3390/antibiotics8010004.
12. Ren H, Liu J. AsnB is involved in natural resistance of *Mycobacterium smegmatis* to multiple drugs. Antimicrob Agents Chemother. 2006;50(1):250-5. doi: 10.1128/aac.50.1.250-255.2006.
13. Bhunu B, Mautsa R, Mukanganyama S. Inhibition of biofilm formation in *Mycobacterium smegmatis* by *Parinari curatellifolia* leaf extracts. BMC Complement Altern Med. 2017;17(1):285. doi: 10.1186/s12906-017-1801-5.
14. Liu Z, Zhuang W, Hu L, Rong R, Li J, Ding W, et al. Experimental and numerical study of potential infection risks from exposure to bioaerosols in one BSL-3 laboratory. Build Environ. 2020;179:106991. doi: 10.1016/j.buildenv.2020.106991.
15. Montgomery MG, Petri J, Spikes TE, Walker JE. Structure of the ATP synthase from *Mycobacterium smegmatis* provides targets for treating tuberculosis. Proc Natl Acad Sci U S A. 2021;118(47):e2111899118. doi: 10.1073/pnas.2111899118.
16. Nyambuya T, Mautsa R, Mukanganyama S. Alkaloid extracts from *Combretum zeyheri* inhibit the growth of *Mycobacterium smegmatis*. BMC Complement Altern Med. 2017;17(1):124. doi: 10.1186/s12906-017-1636-0.
17. Ravindran R, Chakrapani G, Mitra K, Doble M. Inhibitory activity of traditional plants against *Mycobacterium smegmatis* and their action on Filamenting temperature sensitive mutant Z (FtsZ)-a cell division protein. PLoS One. 2020;15(5):e0232482. doi: 10.1371/journal.pone.0232482.
18. Zhang AT, Montgomery MG, Leslie AG, Cook GM, Walker JE. The structure of the catalytic domain of the ATP synthase from *Mycobacterium smegmatis* is a target for developing antitubercular drugs. Proc Natl Acad Sci U S A. 2019;116(10):4206-11. doi: 10.1073/pnas.1817615116.
19. Ayeni EA, Gong Y, Yuan H, Hu Y, Bai X, Liao X. Medicinal plants for anti-neurodegenerative diseases in West Africa. J Ethnopharmacol. 2022;285:114468. doi: 10.1016/j.jep.2021.114468.
20. Nurudeen QO, Ajiboye TO. Aqueous root extract of *Lecaniodiscus cupanioides* restores the alterations in testicular parameters of sexually impaired male rats. Asian Pac J Reprod. 2012;1(2):120-4. doi: 10.1016/s2305-0500(13)60062-7.
21. Ajao AA, Sibiya NP, Moteetee AN. Sexual prowess from nature: a systematic review of medicinal plants used as aphrodisiacs and sexual dysfunction in sub-Saharan Africa. S Afr J Bot. 2019;122:342-59. doi: 10.1016/j.sajb.2018.08.011.
22. Ojo O, Ndinteh DT. Traditional uses, biological activities, and phytochemicals of *Lecaniodiscus cupanioides*: a review. Phys Sci Rev. 2023;8(4):549-65. doi: doi:10.1515/psr-2020-0207.
23. Yemitan OK, Adeyemi OO. CNS depressant activity of *Lecaniodiscus cupanioides*. Fitoterapia. 2005;76(5):412-8. doi: 10.1016/j.fitote.2005.02.010.
24. Ajiboye TO, Nurudeen QO, Yakubu MT. Aphrodisiac effect of aqueous root extract of *Lecaniodiscus cupanioides* in sexually impaired rats. J Basic Clin Physiol Pharmacol. 2014;25(2):241-8. doi: 10.1515/jbcpp-2013-0061.
25. Okore VC, Ugwu CM, Oleghe PO, Akpa PA. Selective anti-candidal action of crude aqueous pod extract of *Lecaniodiscus cupanioides*: a preliminary study on *Candida albicans* obtained from an AIDS patient. Sci Res Essays. 2007;2(2):43-6.
26. Alayande KA, Ashafa AOT. Evaluation of cytotoxic effects and antimicrobial activities of *Lecaniodiscus cupanioides* (Planch.) leaf extract. Trans R Soc S Afr. 2017;72(1):33-8. doi: 10.1080/0035919x.2016.1214851.
27. Nafiu MO, Abdulsalam TA, Akanji MA. Phytochemical analysis and antimalarial activity aqueous extract of *Lecaniodiscus cupanioides* root. J Trop Med. 2013;2013:605393. doi: 10.1155/2013/605393.
28. Encarnación R, Kenne L, Samuelsson G, Sandberg F.

Structural studies on some saponins from *Lecaniodiscus cupanioides*. Phytochemistry. 1981;20(8):1939-42. doi: 10.1016/0031-9422(81)84039-3.
29. Messi LM, Noté OP, Mbing JN, Vansteelandt M, Lavedan P, Vedrenne M, et al. Farnesyl glycosides and one new triterpenoid saponin from the roots of *Lecaniodiscus cupanioides* Planch. ex Benth. Carbohydr Res. 2020;495:108092. doi: 10.1016/j.carres.2020.108092.
30. Adesegun SA, Coker HA, Hamann MT. Anti-cancerous triterpenoid saponins from *Lecaniodiscus cupanioides*. J Nat Prod (Gorakhpur). 2014;7:155-61.
31. Adesegun SA, Coker HA, Hamann MT. Antifungal triterpenoid saponins from *Lecaniodiscus cupanioides*. Res J Phytochem. 2008;2(2):93-9. doi: 10.3923/rjphyto.2008.93.99.
32. Mativandlela SP, Lall N, Meyer JJ. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. S Afr J Bot. 2006;72(2):232-7. doi: 10.1016/j.sajb.2005.08.002.
33. Sun Z, Zhang Y. Antituberculosis activity of certain antifungal and antihelmintic drugs. Tuber Lung Dis. 1999;79(5):319-20. doi: 10.1054/tuld.1999.0212.
34. Ihenetu GU, Igwe OU, Nnaji JC, Ejezie MU. Isolation of 1-eicosene from the leaves of *Baphia nitida*. J Chem Soc Niger. 2020;45(3):434-40.
35. Amen Y, Marzouk AM, Zaghloul MG, Afifi MS. Bioactive compounds from *Tipuana tipu* growing in Egypt. J Am Sci. 2013;9(10):334-9.
36. Kaigongi MM, Lukhoba CW, Yaouba S, Makunga NP, Githiomi J, Yenesew A. In vitro antimicrobial and antiproliferative activities of the root bark extract and isolated chemical constituents of *Zanthoxylum paracanthum* Kokwaro (Rutaceae). Plants (Basel). 2020;9(7):920. doi: 10.3390/plants9070920.
37. Harborne AJ. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. London: Chapman & Hall; 1998. p. 1-36.
38. Nath MC, Chakravorty MK, Chowdhury SR. Liebermann-Burchard reaction for steroids. Nature. 1946;157:103. doi: 10.1038/157103b0.
39. Bohm BA, Koupai-Abyazani MR. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and V. *calycinum* (Ericaceae). Pac Sci. 1994;48:458-63.
40. El Aziz MM, Ashour AS, Gomha Melad AS. A review on saponins from medicinal plants: chemistry, isolation, and determination. J Nanomed Res. 2019;8(1):282-8. doi: 10.15406/jnmr.2019.07.00199.
41. Wardani G, Mahmiah, Sudjarwo SA. In vitro antibacterial activity of chitosan nanoparticles against *Mycobacterium tuberculosis*. Pharmacogn J. 2018;10(1):162-6. doi: 10.5530/pj.2018.1.27.
42. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64(8):711-3. doi: 10.1055/s-2006-957563.
43. Waters M, Tadi P. Streptomycin. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2021. Available from: http://www.ncbi.nlm.nih.gov/books/NBK555886/. Accessed December 2, 2021.
44. Parthasarathy R, Shanmuganathan R, Pugazhendhi A. Vinblastine production by the endophytic fungus *Curvularia verruculosa* from the leaves of *Catharanthus roseus* and its in vitro cytotoxicity against HeLa cell line. Anal Biochem. 2020;593:113530. doi: 10.1016/j.ab.2019.113530.
45. Istifli ES, Hüsunet MT, Ila HB. Cell division, cytotoxicity, and the assays used in the detection of cytotoxicity. In: Cytotoxicity-Definition, Identification, and Cytotoxic Compounds. IntechOpen; 2019. p. 1-9. doi: 10.5772/intechopen.88368.
46. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and medicinal uses of terpenes. In: Joshee N, Dhekney SA, Parajuli P, eds. Medicinal Plants: From Farm to Pharmacy. Cham: Springer; 2019. p. 333-59. doi: 10.1007/978-3-030-31269-5_15.
47. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47. doi: 10.1017/jns.2016.41.
48. Coronado-Aceves EW, Gigliarelli G, Garibay-Escobar A, Zepeda RER, Curini M, López Cervantes J, et al. New Isoflavonoids from the extract of *Rhynchosia precatoria* (Humb. & Bonpl. ex Willd.) DC. and their antimycobacterial activity. J Ethnopharmacol. 2017;206:92-100. doi: 10.1016/j.jep.2017.05.019.
49. Safwat NA, Kashef MT, Aziz RK, Amer KF, Ramadan MA. Quercetin 3-O-glucoside recovered from the wild Egyptian Sahara plant, *Euphorbia paralias* L., inhibits glutamine synthetase and has antimycobacterial activity. Tuberculosis (Edinb). 2018;108:106-13. doi: 10.1016/j.tube.2017.11.005.
50. Kaemchantuek P, Chokchaisiri R, Prabpai S, Kongsaeree P, Chunglok W, Utaipan T, et al. Terpenoids with potent antimycobacterial activity against *Mycobacterium tuberculosis* from *Trigonostemon reidioides* roots. Tetrahedron. 2017;73(12):1594-601. doi: 10.1016/j.tet.2017.02.006.
51. Zhu CZ, Hu BY, Liu JW, Cai Y, Chen XC, Qin DP, et al. Anti-*Mycobacterium tuberculosis* terpenoids from *Resina commiphora*. Molecules. 2019;24(8):1475. doi: 10.3390/molecules24081475.
52. Lozano SA, de Sousa AB, de Souza JC, da Silva DR, Salazar MG, Halicki PC, et al. *Duroia saccifera*: in vitro germination, friable calli and identification of β-sitosterol and stigmasterol from the active extract against *Mycobacterium tuberculosis*. Rodriguésia. 2020;71:e01092019. doi: 10.1590/2175-7860202071054.
53. Fomogne-Fodjo MC, Ndinteh DT, Olivier DK, Kempgens P, van Vuuren S, Krause RW. Secondary metabolites from *Tetracera potatoria* stem bark with anti-mycobacterial activity. J Ethnopharmacol. 2017;195:238-45. doi: 10.1016/j.jep.2016.11.027.
54. Nimbeshaho F, Mwangi C, Orina F, Chacha M, Adipo N, Moody J, et al. Antimycobacterial activities, cytotoxicity and phytochemical screening of extracts for three medicinal plants growing in Kenya. J Med Plants Res. 2020;14(4):129-43. doi: 10.5897/jmpr2020.6905.
55. Pezzuto JM. Natural Compounds in Cancer Therapy. John Boik, Oregon Medical Press, Princeton, MN, 2001, $32.00 (ISBN 0-9648280-1-4). Pharm Biol. 2002;40(1):79. doi.org/10.1076/phbi.40.1.79.5858.
56. Ogbole OO, Segun PA, Adeniji AJ. In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. BMC Complement Altern Med. 2017;17(1):494. doi: 10.1186/s12906-017-2005-8.