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ANTIBACTERIAL EFFECTS AND PHYTOCHEMICAL PROFILING OF ETHNOMEDICINAL PLANT *LECANIODISCUS CUPANIOIDES* PLANCH. EX BENTH FROM SOUTH-WESTERN NIGERIA

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Abstract

Lecaniodiscus cupanioides Planch. ex Benth (Order: Sapindales; Family Sapindaceae) is an ethnomedicinal plant that is used as an effective herbal remedy for human diseases and infections in local communities across Africa and Asia. However, its pharmacologically bioactive constituents remain largely unknown. In the present study, extracts from the leaves part of *L. cupanioides* plant were screened against eleven (11) strains of bacteria by employing microplate broth dilution method. The phytochemical profile of the hexane extract was determined by Gas Chromatography-Mass Spectrometry (GC-MS). The extracts exhibited broad spectrum of antibacterial activities against the tested strains, with minimum inhibitory concentration (MIC) ranging from 0.10 - 3.33 mg/mL. GC-MS analysis of the extract putatively confirmed the presence 48 phytochemicals which could be responsible for the antibacterial activity. These include phytol, β-citronellol, hexadecanoic acid methyl ester, 1-heptacosanol, and neophytadiene among others. However, it is pertinent to isolate, elucidate and unequivocally evaluate the antibacterial activity of the individual bioactive compound, and elucidate their mechanism of antibacterial actions. According to the literature search and to the best of our knowledge, the phytochemical composition of the leaves extract from *L. cupanioides*, investigated by GC-MS, was studied for the first time in this study. The current study justifies the clinical traditional uses of *L. cupanioides* in the management of diseases and infections caused by pathogenic bacteria in Nigerian ethnomedicine.

Keywords: *Medicinal plant, Lecaniodiscus cupanioides, Antibacterial, Phytochemicals, 1-heptacosanol*

Introduction

The use of plants and herbs as medicines in different countries is as old as the existence of man, and they have been regarded as indispensable source of therapeutically valued phytochemical compounds (1,2). These naturally-derived chemical compounds are used in traditional medicine to combat various forms of human infections and diseases with little or no side effects (3,4). This development has been initiated by the increase in the resistance of microbial agents, especially the pace of evolution of pandrug-resistant bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumonia* and other members of "ESKAPE" human pathogens to the current antibiotics, which has become a persistent global threat (5,6). Although a range of synthetic antibiotics is currently being used to treat human bacterial infections, these synthetic drugs always cause adverse effects in the human body (7,8). Therefore, there is an urgent need to discover lead drug candidates from the medicinal plants source to benefit mankind.

Lecaniodiscus cupanioides Planch. ex Benth (Sapindaceae) is an ethnomedicinal plant that is indigenous to mainly Africa and Asia. Ethnobotanical surveys on the plant herbal therapy indicated that *L. cupanioides* is used in the clinical traditional medicine in South-Western Nigeria for the treatment of a wide range of human sickness and diseases, such as cough, jaundice (9), skin infections, malaria (10), wounds (11), sexual dysfunction, cancer (12), diabetes, typhoid (13,14), and infant illness (15). Previous pharmacological studies have shown that *L. cupanioides* possesses cytotoxic (16), antidiabetic (17) and sexual prowess (18) properties. Similarly, previous phytochemical investigations have identified antifungal and anticancer triterpenoid saponins from the stem-bark of the plant (19–21), while Messi et al. (22) in a recent study isolated cupanioidesosides A, B and C, together with lecanioside A from the plant root extract. At present, the phytochemicals of *L. cupanioides* leaves are yet not comprehensively studied. With this background, we investigated the antibacterial effects of *L. cupanioides* leaves extracts. Furthermore, the leaf extract was subjected to gas chromatography coupled with mass spectrometer (GC-MS), for the first time, to profile its phytochemical constituents, which might be responsible for its antibacterial activity. Findings from this study may provide basis for further research involving drug-resistant strains of the test organisms.

Methods

Plant collection

The fresh *L. cupanioides* leaves sample was collected from the University of Ibadan, Oyo State, Western Nigeria (Geographical location: 7° 23' 28N 3° 54' 60E) during the month of September, 2019. The plant sample was identified and authenticated taxonomically by Mr. D. P. O. Esimekhuai, a Chief Plant Technologist at the Botany Department, University of Ibadan, Nigeria. The voucher specimen, UIH-22898, has been deposited in the same department. The detached leaves sample was thoroughly washed and air-dried for two weeks in an open shaded place at room temperature. The air-dried leaves were milled into fine powder with the aid of an industrial grinder at the Wood Extraction Laboratory, Department of Chemistry, University of Ibadan, Nigeria and kept in a sealed polythene bag until further use at room temperature.

Extraction of plant material

Plant extracts preparation was carried out at the Chemical Sciences Department, University of Johannesburg, South Africa using the methods of Mathekga and Meyer (23) with little modifications. Briefly, 250 g of milled leaves sample was soaked and extracted repeated (5 times) with n-hexane (1 L), chloroform (1 L), and butanol:water (1:1, v/v) respectively for 24 h. With varying degree of solvents polarity (non-polar – moderate polar – polar solvent), we anticipated to track down the extracts that may be accountable for the antibacterial activity. The plant samples were filtered with the aid of Whatman No. 1 filter paper. This was followed by concentration of the filtrates to dryness by means of a rotary evaporator under reduced pressure at 40 °C. The concentrated crude extracts that were obtained accordingly were kept at the room temperature until needed for further studies.

Determination of antibacterial activity

Chemicals for antibacterial bioassay

Dimethyl sulfoxide (DMSO), Muller-Hilton broth, Streptomycin and nalidixic acid (Sigma-Aldrich)

Microbial cultures

The micro-organisms used for the antibacterial investigation of the hexane, chloroform, and butanol:water crude extracts of *L. cupanioides* in this study were selected because of their clinical importance. These include standard strains of six Gram-negative bacteria species, namely *Escherichia coli* (**ATCC 25922**), *Enterobacter cloacae* (**ATCC 13047**), *Proteus mirabilis* (**ATCC 7002**), *Klebsiella aerogenes* (**ATCC 13882**), *Pseudomonas aeruginosa* (**ATCC 27853**) and *Klebsiella oxytoca* (**ATCC 8724**); standard strains of four Gram-positive bacteria strains, namely *Bacillus subtilis* (**ATCC 19659**), *Enterococcus faecalis* (**ATCC 13047**), *Staphylococcus aureus* (**ATCC 25923**), *Staphylococcus epidermidis* (**ATCC 14990**); and a fast-growing *Mycobacterium* strain, *Mycobacterium smegmatis* (**MC 2155**). These standard strains were obtained from Biotechnology and Food Technology Department of the University of Johannesburg, South Africa, and maintained at the microbiology laboratory of the same department at 4 °C. The pure cultures of bacteria strains, under hygienic conditions, were sub-cultured onto Muller-Hilton Agar (MHA).

Antibacterial test

The antibacterial activities of the three extracts were tested *in vitro* against the eleven bacteria strains using microdilution method (24). The minimum inhibitory concentrations (MICs) were determined according to standard procedure (25). Streptomycin and Nalidixic acid were used as positive control to compare the activity of the extracts with known antibacterial drugs. Muller-Hilton broth (50% v/v in DMSO) served as the negative control. Briefly, 10 mg of each of the plant extracts were dissolved in 3mL of DMSO to prepare the sample stock solutions. In a 96-well plate containing one hundred microliters (100 µL) of serially diluted (two-fold dilution) test samples concentration of 3.33, 1.67, 0.83, 0.42, 0.21 and 0.10 mg/mL, one hundred microliters (100 µL) of standardized suspension of inoculums were seeded in duplicate under aseptic conditions (Laminar flow unit). The plates were sealed with sterile seals and then incubated at 37 °C for 24 h. Viable bacterial cells were confirmed calorimetrically by adding resazurin dye (40 µL of 200 µg/mL) after 2 h incubation as they enzymatically reduced resazurin dye (blue colour) to the resorufin (pink colour), and remained blue in death cells. The concentration of DMSO in the well had no effect on the bacterial growth. The smallest concentration that irreversibly converted the blue dye into pink and exhibited total inhibition of the growth of bacteria was used as the MIC (26), and the values are reported for each tested extract together with the standards as shown in **Table 1**.

GC-MS analysis

The hexane crude extract was subjected to GC-MS analysis after dissolving in HPLC-grade methanol and filtering through a 0.2 µm PTFE syringe-driven filter into GC-MS vial. **Table 2** depicts the experimental conditions for the GC-MS analysis. The identification of the phytochemicals present in the extract was achieved by comparing their spectra with the spectral fingerprint of the known compounds in the curated database of National Institute Standard and Technology (NIST). Consequently, the names, the molecular formulae, the molecular weight as well as the chemical structures of the phytochemicals in the analyzed extract were identified. Relative quantity of the phytochemicals presents in the hexane leaves extract of *L. cupanioides* was expressed as percentage (%) based on peak area produced in the chromatogram.

Results

The results of antibacterial analysis of the tested extracts of *L. cupanioides* against the bacteria strains are depicted in **Table 1**. The findings showed that the studied plant extracts displayed varying degree of antibacterial activity against the tested strains, with MICs ranging from 0.10 to 3.33 mg/mL. The tested strains of the bacteria reacted differently to the plant extracts and their susceptibility was concentration-dependent. The chloroform extract showed significant activity against *B. subtilis*, *K. aerogenes* and *E. coli*, with a MIC value of 0.10

mg/mL. *P. aeruginosa*, one of the members of "ESKAPE" human pathogens knows for its multidrug resistance, is sensitive to all the plant extracts investigated in this study with MIC value of 1.67 mg/mL. Nalidixic acid (NLD), one of the reference antibiotics used, showed different inhibitory activity against the bacteria with varying MIC values (0.008 to 0.512 mg/mL), and in some cases its MICs were lower than those obtained from the chloroform extract on *E. coli* and *K. aerogenes*. Streptomycin (STM) produced MIC values ranging from 0.004 to 0.512 mg/mL, and there was no activity recorded against negative control (50% broth in DMSO, v/v).

Furthermore, GC-MS profiling of the hexane extract revealed a total of 60 peaks of which 48 phytochemical compounds belonging to different chemical families were identified. The peak number, retention time (RT), peak area (%), formulae, and molecular weight of the identified compounds are documented in **Table 3**. GC-MS chromatogram of the hexane extract of *L. cupanioides* was shown in Fig. 1. The most abundant compounds observed included (E)-9-octadecenoic acid methyl ester (18.42%), 2-hexadecen-1-ol-3,7,11,15-tetramethyl (7.39%), hexadecanoic acid methyl ester (7.10%), nonanoic acid 9-oxo-methyl ester (6.26%), octadecanoic acid methyl ester (5.62%), 1-nonadecene (4.41%), 14-Beta-H-Pregna (4.22%), 1-heptacosanol (3.93%) and octadecyl trifluoroacetate (3.83%). Similarly, majority of the identified constituents are fatty acids or their ester derivatives. Some of the structures of the biologically active phytochemicals identified from *L. cupanioides* were shown in Fig. 2.

Discussion

Infectious diseases caused by bacterial agents, such as *P. aeruginosa*, *S. aureus*, *E. coli*, *P. mirabilis*, and *K. aerogene* remain a heavy burden in Africa and the world in general. This is worsening by the emergent of tuberculosis as these pathogenic organisms facilitate the infection by *Mycobacterium tuberculosis* or significantly weaken the immune system for other opportunistic infections, such as Human Immunodeficiency Virus/Acquired Immune Deficiency Syndromes (HIV/AIDS). Nowadays, most of these microorganisms have acquired one form of resistant mechanism or the other against most of the current clinical antibiotics, leading to treatment failure or longer treatment option with increased health care costs, and at its worst mortality or morbidity. Plant extracts are promising sources of antibacterial agents as several studies have shown the anti-infective activities of secondary metabolites present in plant extracts (27,28). In view of this, three extracts of *L. cupanioides* were studied for various activities against infective agents. The extracts investigated in this study exerted a broad spectrum of antibacterial activity by effectively inhibiting the growth of all the Gram-negative and Gram-positive bacteria strains as well as *Mycobacterium smegmatis*. Interestingly, B. *subtilis*, *K. aerogenes* and *E. coli* were highly susceptible to the chloroform extract. Clinically, Gibbons (29) was of the opinion that a plant extract or its isolated phytochemical has little or no relevance if its MIC value is > 1 mg/ml. Therefore, it follows that chloroform leaves extract can be considered as a good source of antibacterial agents against *E. coli* and *K. aerogenes* based on its MIC value of 0.1 mg/mL, which is lower than that of the Nalidixic acid (Reference antibiotic) used.

Gram-negative bacteria, such as *P. aeruginosa* and *E. coli* are causative agents of bacteremia and serious gastrointestinal infections, such as diarrhoea and dysentery. These Gram-negative bacteria are reportedly resistant to antibiotics (30–32). Moreover, infections caused by Gram-negative pathogens are more difficult to treat due to their highly restrictive permeability membranes to most antibiotics (33). However, susceptibility of the above pathogens to the studied extracts suggested that the extracts are worthy of further investigation as an antibacterial agent. The antibacterial efficacy of these extracts against these pathogens, therefore, justify their use in the treatment of diseases, such as diarrhoea, dysentery and other similar infections. *S. aureus*, a versatile and dangerous Gram-positive human bacterium, is the causative agent of multiple human infections, such as skin and soft tissues infections, fever, gastroenteritis, pneumonia, urinary tract infections, bacteremia and septic arthritis depending on the site of infections (34). Recently, WHO categorized it among the drug-

resistant pathogens that need to be critically prioritized for global human health (35). Of all the three extracts investigated in this study, hexane and chloroform extract showed better activity against *S. aureus* (MIC = 1.67 mg/mL) compared to butanol-water extract.

The antibacterial activities of *L. cupanioides* using hexane, ethanol (36), methanol (37), methanol-water (13) and water (38) as solvents for extraction have been previously reported in the literature. Thus, the findings from this study corroborates with reports of previous work on the plant. However, there are no reports, to date, in the literature on the GC-MS-based metabolite profiling of extracts of *L. cupanioides* to detect the presence of various phytochemical compounds, which could be explored as antibacterial agents. Therefore, GC-MS analysis was carried out in the present study and led to the identification of 48 phytochemicals from the hexane leaves extract. Most of the identified compounds are known to exhibit antibacterial activity which could be linked to the most abundant bioactive phytochemicals, including (E)-9-octadecenoic acid methyl ester, 2-hexadecen-1-ol-3,7,11,15-tetramethyl, hexadecanoic acid methyl ester, nonanoic acid 9-oxo-methyl ester, and octadecanoic acid methyl ester and other phyto-compounds (39,40). Hexadecanoic acid methyl ester is a major metabolite in many herbal plants. It was reported to show inhibitory activity against clinical pathogenic bacteria (41). Similarly, phytol which is a diterpene has been reported to possess antibacterial activity. It mechanistically induced oxidative cell death in *P. aeruginosa* (42,43). In addition, it has been shown to inhibit the growth of *Mycobacterium tuberculosis* (44). 1-heptacosanol is a long-chain fatty alcohol with reported antimicrobial activity against *E. coli* and *S. aureus* (45). Unlike compound β-citronellol which is also reported with various biological activities, including antibacterial (46,47), 3-ethyl-6-trifluoroacetoxyoctane has not yet been demonstrated for antibacterial activity but its presence has been profiled in other plant extract with antibacterial and anticancer activities (48). Neophytadiene, apart from having antioxidant and anti-inflammatory activity, is considered as an antibacterial agent (49,50). Trans-10-methyl-4-ketoperhydroazulene detected in the extract is a synthetic compound (51). There is no evidence in the available literature that it is a metabolite produced by plants and/or that it is pharmacologically active. Therefore, trans-10-methyl-4-ketoperhydroazulene might be probably incorporated into the *L. cupanioides* tissue from the external source. Some other phytochemicals, including tetracontane, heptadeca-7,10-dione, hexadecane, and 2-undecenal have not been described in detail in the literature. However, further studies are needed to isolate and characterize these phytochemicals from *L. cupanioides*, and assess their antibacterial activity in order to ascertain their therapeutic values.

Conclusions

In this study, hexane, chloroform and butanol:water leaves extracts of *L. cupanioides* exhibited broad spectrum of activity against both the Gram-positive and the Gram-negative strains of bacteria. The GC-MS analysis of the hexane extract led to the identification of 48 bioactive phytochemicals, which greatly contributed to its antibacterial activity. This present study is the first report on the GC-MS metabolic profiling of *L. cupanioides*. These results clearly justified the traditional clinical uses of *L. cupanioides* for the treatment of infections and diseases caused by pathogenic bacteria. However, it is pertinent to isolate, characterize and unequivocally evaluate the antibacterial activity of the individual bioactive compound, and elucidate their mechanism of actions.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Table 1. Minimum inhibitory concentration (MIC) of the studied extracts against the tested bacterial strains

Minimum inhibitory concentration (mg/mL)											
Test extract code	***Gram-positive***				***Gram-negative***						
	Bs	***Ef***	***Se***	***Sa***	***Ms***	***Ecl***	***Ko***	***Ka***	***Pm***	***Ec***	***Pa***
LCB/W	0.83	1.67	1.67	3.33	0.83	1.67	1.67	1.67	1.67	1.67	1.67
LCC	0.10	3.33	1.67	1.67	1.67	3.33	1.67	0.10	3.33	0.10	1.67
LCH	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	3.33	1.67	1.67
STM	*0.016*	*0.128*	*0.008*	*0.256*	*0.004*	*0.512*	*0.016*	*0.016*	*0.128*	*0.064*	*0.512*
NLD	*0.016*	*0.512*	*0.064*	*0.064*	*0.512*	*0.016*	*0.008*	*0.256*	*0.032*	*0.512*	*0.256*

Bacteria strains*: Bs - Bacillus subtilis*; *Ef - Enterococcus faecalis*; *Se - Staphylococcus epidermidis*; *Sa - Staphylococcus aureus*; *Ms - Mycobaterium smegmatis*; *Ecl - Enterobacter cloacae*; *Ko - Klebsiella oxytoca*; *Ka -* Klebsiella *aerogenes*; *Pm - Proteus mirabilis*; *Ec - Escherischia coli*; *Pa - Pseudomonas aeruginosa*; **Extracts**: LCB/W - *Lecaniodiscus cupanioides* Butanol/Water Extract; LCC - *Lecaniodiscus cupanioides* Chloroform Extract; LCH - *Lecaniodiscus cupanioides* Hexane Extract; **Drugs**: STM – Streptomycin; NLD – Nalidixic acid

Table 2. The experimental conditions for the GC–MS analysis

GC conditions	
Equipment	Agilent Technologies (GC-7890B: MS-5977AMSB)
Detector	Mass detector
Column	HP-5MS (5 % phenyl methyl siloxane), 30 m 9 250 µm 9 0.25 µm
Column oven temperature	50 °C to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 300 °C at 10 °C/min
Sample injection	1 μL
Carrier gas	Helium gas (99.9%) 1 ml/min, splitless mode
Injector temperature	290 °C
Software	MassHunter
GC running time	19 min
MS conditions	
Library used	NIST version—2014
Electron energy	70 eV
Source temperature	350 °C
Inlet line temperature	250 °C
Solvent delay	10 mins
Mass scan (m/z)	40 to 1000 amu

Table 3. Phytochemicals detected with GC–MS profiling of the hexane leaves extract of *Lecaniodiscus cupanioides*

S/N	Phytochemical name	Peak number	Retention time	Peak area (%)	Molecular formula	Molecular weight	Compound nature
1	4-oxononanal	1	10.019	0.38	$C_9H_{16}O_2$	156.22	Medium-chain aldehyde
2	Dodecane	2	10.612	0.34	$C_{12}H_{26}$	170.33	Straight-chain alkane
3	Methyl-4-oxo-octanoate	3	11.012	0.76	$C_9H_{16}O_3$	172.22	Medium-chain ester
4	2-Undecenal	4	11.369	0.21	$C_{11}H_{20}O$	168.28	Medium-chain aldehydes
5	Tetradecane	5	11.693	0.49	$C_{14}H_{30}$	198.39	Straight-chain alkane
6	Octanoic acid, 8-hydroxy-, methyl ester	6	11.725	0.33	$C_9H_{18}O_3$	174.24	Medium-chain ester
7	Nonanoic acid, 9-oxo-, methyl ester	7	12.092	6.26	$C_{10}H_{18}O_3$	186.25	Medium-chain ester
8	Nerylacetone	8	12.265	0.37	$C_{13}H_{22}O$	194.31	Nor-monoterpene ketone
9	Hexadecane	9	12.664	0.74	$C_{16}H_{34}$	226.44	Straight-chain alkane
10	Decanoic acid,9-oxo-, methyl ester	10	12.896	0.42	$C_{11}H_{20}O_3$	200.27	Medium-chain ester
11	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-	11	13.195	0.21	$C_8H_{10}O_2$	138.16	Heterocyclic hydrocarbon
12	9-Eicosene, (E)-	12	13.497	1.38	$C_{20}H_{40}$	280.5	Unsaturated aliphatic hydrocarbon
13	Methyl 12-oxo-9-dodecenoate	15	14.446	0.35	$C_{13}H_{22}O_3$	226.31	Medium-chain ester
14	1-Hexadecanol, 3,7,11,15-tetramethyl-	16	14.642	0.23	$C_{20}H_{40}O$	298.5	Medium-chain ester
15	13-Methylpentadec-14-ene-1,13-diol	17	14.716	0.26	$C_{16}H_{32}O_2$	256.42	Medium-chain alcohol
16	3-Ethyl-6-trifluoroacetoxy octane	18	14.835	0.14	$C_{12}H_{21}F_3O_2$	254.29	
17	1-Nonadecene	19	15.132	3.36	$C_{19}H_{38}$	266.5	Medium-chain hydrocarbon
18	Neophytadiene	22	15.494	1.19	$C_{20}H_{38}$	278.5	Diterpene
19	2-Pentadecanone,6,10,14-trimethyl-	23	15.560	2.29	$C_{18}H_{36}O$	268.5	Sesquiterpenoid

20	Carbonic acid, eicosyl vinyl ester	26	15.925	0.68	$C_{23}H_{44}O_3$	368.6	Ester
21	Hexadecanoic acid, methyl ester	27	16.135	7.10	$C_{17}H_{34}O_2$	270.5	Fatty acid ester
22	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	28	16.485	0.36	$C_{18}H_{24}O_4$	304.4	Ester
23	3-Dodecanol, 3,7,11-trimethyl-	29	16.542	0.51	$C_{15}H_{32}O$	228.41	Fatty alcohol
24	β-Citronellol	31	16.702	0.42	$C_{10}H_{20}O$	156.26	Monoterpenoid
25	Heptadecanoic acid, methyl ester	32	16.832	0.19	$C_{18}H_{36}O_2$	284.5	Fatty acid ester
26	9-Octadecenoic acid, methyl ester	33	17.384	18.42	$C_{19}H_{36}O_2$	296.48	Fatty acid ester
27	2-Hexadecen-1-ol, 3,7,11,17-tetramethyl (Phytol)	34	17.473	7.39	$C_{20}H_{40}O$	296.5	Diterpene alcohol
28	Octadecanoic acid, methyl ester	35	17.517	5.62	$C_{19}H_{38}O_2$	298.5	Fatty acid ester
29	2-Pentylcyclopentanone	36	17.670	0.64	$C_{10}H_{18}O$	154.25	Cyclic ketone
30	Phytol, acetate	37	17.706	0.66	$C_{22}H_{42}O_2$	338.6	Diterpene ester
31	1-Octanol, 3,7-dimethyl-	38	17.820	0.15	$C_{10}H_{22}O$	158.28	Aliphatic alcohol
32	Hexadecanoic acid, butyl ester	39	17.895	0.63	$C_{20}H_{40}O_2$	312.53	Fatty acid ester
33	Octadecyl trifluoroacetate	40	17.935	3.83	$C_{20}H_{37}F_3O_2$	366.50	Ester
34	Propanoic acid, 2-methyl-, butyl ester	41	18.068	0.46	$C_8H_{16}O_2$	144.21	Fatty acid ester
35	Oxiraneoctanoicacid, 3-octyl-, methyl ester	43	18.517	3.48	$C_{19}H_{36}O_3$	312.5	Fatty acid ester
36	Vinyl caprylate	44	18.577	1.40	$C_{10}H_{18}O_2$	170.25	Ester
37	14-Beta-H-Pregna	46	18.667	4.22	$C_{21}H_{36}$	288.51	Steroid
38	Docosa-2,6,10,14,18-pentaen-22-al,2,6-10,15,18-pentamethyl-	47	18.725	0.37	$C_{27}H_{44}O$	384.6	
39	Methyl 18-methylnonadecanoate	48	18.775	0.85	$C_{21}H_{42}O_2$	326.55	Fatty acid ester
40	Cyclohexadecane	49	18.911	0.57	$C_{16}H_{32}$	224.42	Cyclic hydrocarbon
41	2-Butyl-3-methyl-5-(2-methylprop-2-enyl) cyclohexanone	50	18.988	2.43	$C_{15}H_{26}O$	222.37	Aliphatic ketone

42	4,8,12,16-Tetramethylheptadecan-4-olide	51	19.029	1.86	$C_{21}H_{40}O_2$	324.54	
43	Cyclohexanemethanol, 4-hydroxy-α, α, 4-trimethyl-	52	19.080	0.24	$C_{10}H_{20}O_2$	172.26	Cyclic alcohol
44	1-Heptacosanol	53	19.157	3.93	$C_{27}H_{56}O$	396.73	Long chain fatty alcohol
45	Heptadeca-7,10-dione	55	19.485	0.45	$C_{17}H_{32}O_2$	268.5	Medium-chain ketone
46	Trans-10-methyl-4-ketoperhydroazulene	56	19.528	0.89	$C_{11}H_{18}O$	166.26	
47	9-octadecenoic acid, 1,2,3-propanetriyl ester	57	19.688	0.79	$C_{57}H_{104}O_6$	885.4	Long chain fatty ester
48	Docosanoic acid, methyl ester	60	19.937	0.39	$C_{23}H_{46}O_2$	354.61	Medium chain fatty acid ester

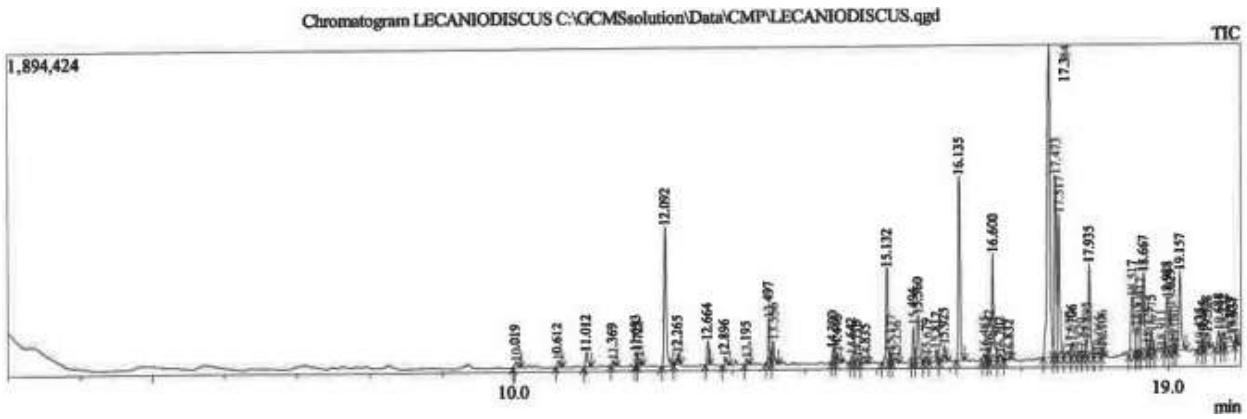
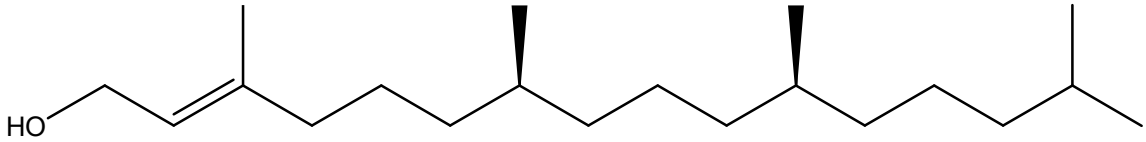
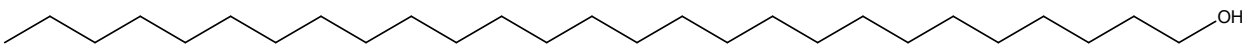


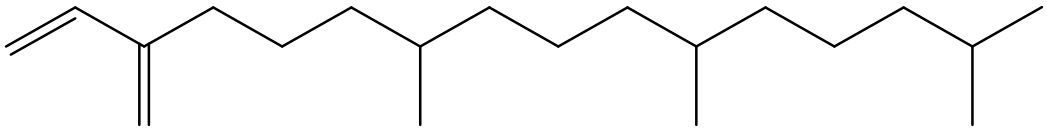
Figure 1. GC–MS chromatogram of the hexane leaves extract from *Lecaniodiscus cupanioides*



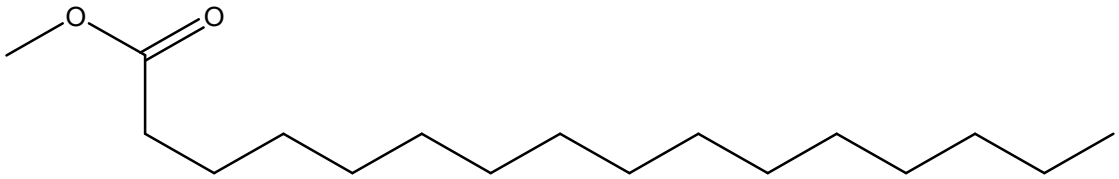
Phytol (**27**)



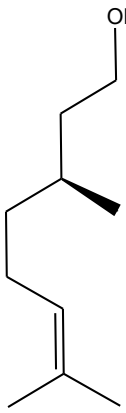
1-Heptacosanol (**44**)



Neophytadiene (**18**)



Hexadecanoic acid, methyl ester (**21**)



β-Citronellol (**24**)

Figure 2. Some structures of pharmacologically active phytochemicals identified from *Lecaniodiscus cupanioides*