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CHEMICAL COMPOSITION PROFILING AND ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OIL AND PLANT EXTRACTS OF *MESEMBRYANTHEMUM EDULE* (L.) BOLUS LEAVES

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Abstract

Background: Essential oil from *Mesembryanthemum edule* leaves have been used by the Eastern Cape traditional healers for the treatment of respiratory tract infections, tuberculosis, dysentery, diabetic mellitus, laryngitis and vaginal infections. The investigation of bioactive compounds in the essential oil of this plant could help to verify the efficacy of the plant in the management or treatment of these illnesses.
Materials and methods: Various concentrations of the hydro-distilled essential oil, ranging from 0.005-5 mg/ml, were tested against some fungal strains, using the micro-dilution method. Minimum inhibitory activity was compared with four other different crude extracts of hexane, acetone, ethanol and aqueous samples from the same plant. The chemical composition of the essential oil, hexane, acetone and ethanol extracts was determined using GC-MS.
Result: GC/MS analysis of the essential oil resulted in the identification of 28 compounds, representing 99.99% of the total oil. Phytoconstituents of hexane, acetone and ethanol extracts yielded a total peak chromatogram of fifty nine compounds. A total amount of 10.6% and 36.61% of the constituents were obtained as monoterpenes and oxygenated monoterpenes. Sesquiterpene hydrocarbons (3.58%) were relatively low compared to the oxygenated sesquiterpenes (9.28%), while the major concentrated diterpenes and oxygenated diterpenes were 1.43% and 19.24 %, respectively and phytol 12.41%. Total amount of fatty acids and their methyl esters content, present in the oil extract, were found to be 19.25 %. Antifungal activity of the oil extract and four solvent extracts were tested against five pathogenic fungal strains. The oil extract showed antifungal activity against *Candida albican*, *Candida krusei*, *Candida rugosa*, *Candida glabrata* and *Cryptococcus neoformans* with MIC ranges of 0.02-0.31 mg/ml. Hexane extract was active against the five fungal strains with MICs ranging between 0.02-1.25 mg/ml. Acetone extracts were active against *C. krusei* only at 0.04mg/ml. No appreciable antifungal activity was found in either ethanol or water extracts when compared with commercial antibiotics.
Conclusion: The profile of chemical constituents found in *M. edule* essential oil and its antifungal properties support the use of *M. edule* by traditional healers as well as in the pharmaceutical and food industries as a natural antibiotic and food preservative.

Key words: Mesembryanthemum edule, Essential oil, GC/MS, Antifungal activity, Opportunistic fungi

Introduction

The global epidemic of HIV/AIDS appears to have stabilized in most regions. However, Sub-Saharan Africa remains a heavily affected region according to the report of UNAIDS and WHO. (2009). Among the Sub-Saharan African countries, South Africa carries the largest burden of HIV/AIDS with an estimated infection rate of 5.38 million out of 50.6 million indigenes in 2011 (Otang et al., 2011). Majority of people living with HIV/AIDS are vulnerable to developing fungal infections because of their suppressed immune systems (Zarrin and Mahmoudabadi, 2009). Fungal infections remain a significant cause of gastrointestinal disease; a consequence of HIV/AIDS, especially in immune-compromised individuals (Otang et al., 2011).The incidence of recurring fungal infection associated with HIV/AIDS has increased dramatically. *Candida albicans* is one of the major causes of mucosal and bloodstream infections (Noble and Johnson, 2007). *Cryptococcus neoformans* is a facultative organism that is very unique in attacking the lymphocytic cells. Meningitis and lung infections are commonly found with *C. neoformans* infections (Goldman et al., 2011).

Candida glabrata currently ranks the second to third causative agent of oral, vaginal, or urinary infections, which is often known as nosocomial disease (Zarrin and Mahmoudabadi, 2009). Mortality rates in compromised patients are very difficult to treat, especially with a fluconazole drug (Hernandez et al., 2004). Susceptibility to invasive candidiasis has increased in populations with suppressed immunological defences, such as those with HIV/AIDS, with *Candida rugosa* emerging in recent years as a distinct cause of candidiasis in trauma patients (Zarrin and Mahmoudabadi, 2009; Behera et al., 2010). *Candida krusei* is ranked as the fifth most common fungal species seen in immune-compromised patients (Behera et al., 2010).

Over the years, the prevalence of fungal infections and their resistance to antibiotic drugs has increased the need for research in alternative treatments against fungal infections. It is noteworthy that researchers have directed their attention towards medicinal plants to develop better drugs against fungal infections. Traditional medicines have played an important role in health services around the globe, especially in South Africa, due to a wide array of phyto-chemicals found in plants with therapeutic properties (Juneja et al., 2012). Owing to this, a large majority of the South African population relies heavily on the use of plants and plant extracts for their wellbeing. Much attention has been drawn to plant-derived fungicides in recent years as a replacement for modern drugs (Stein et al., 2006). It is also reported that the number of individuals using essential oils obtained from plants are less likely to contract infectious diseases (Siveen and Kuttan, 2010). Moreover, essential oil users who eventually contract an infectious disease tend to recover faster than those using antibiotics (Jin-Hui et al., 2013).

In South Africa, essential oils are usually used to preserve food products against the growth of fungi or bacteria. Due to increased demand many of these essential oils from medicinal plants are cheaply distributed and sold in local market centres (Juneja et al., 2012). The high reliance on medicinal plants for health purposes necessitates the scientific validation of their therapeutic value and safety.

Mesembryanthemum edule is an edible ground-cover plant commonly found in the coastal districts of the Eastern Cape of South Africa. The Xhosa-speaking people in this province usually administer alcohol and aqueous extracts to patients for the management of diseases commonly associated with HIV/AIDS (Omoruyi et al., 2012). Based on the ethno-medical information on this plant, four different extracts (hexane, acetone, ethanol and aqueous) of *M. edule* were screened for activity against *Candida albican*, *Candida rogusa*, *Candida krusei*,

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Candida glabrata and *Cryptococcus neoformans*. The activities of *Mesembryanthemum edule* on mycobacteria causing tuberculosis (TB) have been described (Buwa and Afolayan, 2009), but reports on the biological effect of its essential oil on pathogenic fungal strains found in HIV/AIDs patients are limited. Essential oils and their components in this study were observed to be more active against the five fungal strains than the four solvent extracts, which justifies their use as complementary and alternative medicines.

Materials and methods

Plant material

Fresh leaf material of *M. edule* was supplied by an herbalist from Nkonkobe Municipality. The taxonomical identity of the plant was confirmed by a botanist, Prof. DS Grierson, and a voucher specimen was kept in the Griffin Herbarium of the Botany Department, University of Fort Hare as (Omo 2011/1-Omo 2011/19) (Omoruyi et al., 2012) .

Preparation of the extracts

The collected leaves were thoroughly washed with distilled water, chopped and oven dried at approximately 40^{o}C for 48 hours and ground into fine powder. Two hundred and fifty grams of leaf powder were extracted on an orbital shaker for 48 hours, with 2 litres of hexane, acetone, ethanol or distilled water, respectively. The extracts were filtered through a Buchner funnel and Whatman No. 1 filter paper. The filtrate was re-filtered through sterile cotton wool. The filtrates from hexane, acetone and ethanol were evaporated to dryness under reduced pressure using a rotary evaporator at 50^{o}C while the aqueous filtrate was freeze-dried. The yields of hexane (12g), acetone (18g), ethanol (8.4g) and water (7.6g) extracts were recorded. Each extract was re-suspended in their respective solvents to give the required concentration needed for this study and were used immediately.

Essential oil

Volatile oil from the fresh leaves (1000g) was extracted for 3 hrs using a hydro-distiller (Clevenger's-type apparatus) in a 5-L round bottom flask fitted with a condenser.

Gas chromatography–mass spectroscopy analysis

The volatile oil extract was subjected to GC-MS analysis for identification of components in the department of Botany, University of Fort Hare. This was carried out using a GC-MS (HP 6890) with a mass selective detector (HP5973).Identification of the chemical components of the essential oils was accomplished by marching their mass spectra and retention indices with those of the Wiley 275 library (Okoh et al., 2010). The quantity of compounds was calculated by integrating the peak areas of the spectrograms. A needle with the sample material (essential oils tested) was inserted directly into the inlet of the Gas Chromatograph. The initial temperature 70^{o}C, maximum temperature 325^{o}C, equilibration time 3 min, ramp 4^{o}C/min, final temperature 240 ^{o}C; inlet: split less, initial temperature 220^{o}C, pressure 8.27 psi, purge flow 30 ml/min, purge time 0.20 min, gas type helium; column: capillary, 30 m x 0.25mm i.d., film thickness 0.25 µm, initial flow 0.7 ml/min, average velocity 32 cm/s; MS: El method at 70 eV.

Calculation of oil yield

Prior to the final extraction and obtaining the oil, a clean bottle of known mass was prepared. At the end of the extraction process, the oil obtained was carefully transferred into the bottle and the final mass noted. The yield obtained was calculated as follows: Mass of plant material distilled (g) = X; Mass of empty bottle (g) = A; Mass of bottle + oil extracted (g) = B; Mass of oil (g) = (B-A); Percentage (%) yield = [(B-A) ÷ X] 100. The oil was diluted in methanol (20% v/v) and a working concentration ranging between 0.005-5 mg/ml was used for the determination of Minimum Inhibitory Concentration (MIC).

Microorganisms and growth media

The fungi employed in this study were selected mainly on the basis of their importance as common pathogens in humans infected with HIV/ AIDS. Strains used for this study were from the American type culture collection (ATCC). These included *Candida albicans* ATCC 2091, *Candida krusei* ATCC 204305, *Candida glabrata* ATCC 2001, *Candida rugosa* ATCC 10571 and *Cryptococcus neoformans* ATCC 66031. Sabouraud dextrose (SDA) and Sabouraud dextrose broth (SDB) were prepared according to the manufacturer's instructions.

Each fungus was grown for 48 hours at 28 °C in Sabouraud Dextrose Agar (Merck) plates. Scrape cell mass was transferred from each solid culture to 3 ml saline solution and then adjusted to 0.5 Mc Farland standard, which was confirmed by spectrophotometric reading at 580 nm (Duart et al., 2005). Cell suspensions were finally diluted to 10^{4} CFU/ml for the use in the assays.

Minimum Inhibitory Concentration (MIC)

A 96 well-plate micro-dilution method was employed, using Sabouraud dextrose broth as described by Otang et al. (2011). Different concentrations of the diluted extract, ranging from 0.005-5mg/ml, were prepared in the wells in a total volume of 100 µl and 20 µl of 0.5 Mc-Farland fungal suspensions were inoculated into the wells except those which contained sterile distilled water (blank). All treatments were performed in duplicate. The growth of the fungi was determined by measuring the absorbance at 620 nm. The plates were incubated at 37^{0}C for 24 hours. The lowest concentration which inhibited the growth of the fungi was considered the minimum inhibitory concentration (MIC) of each extract.

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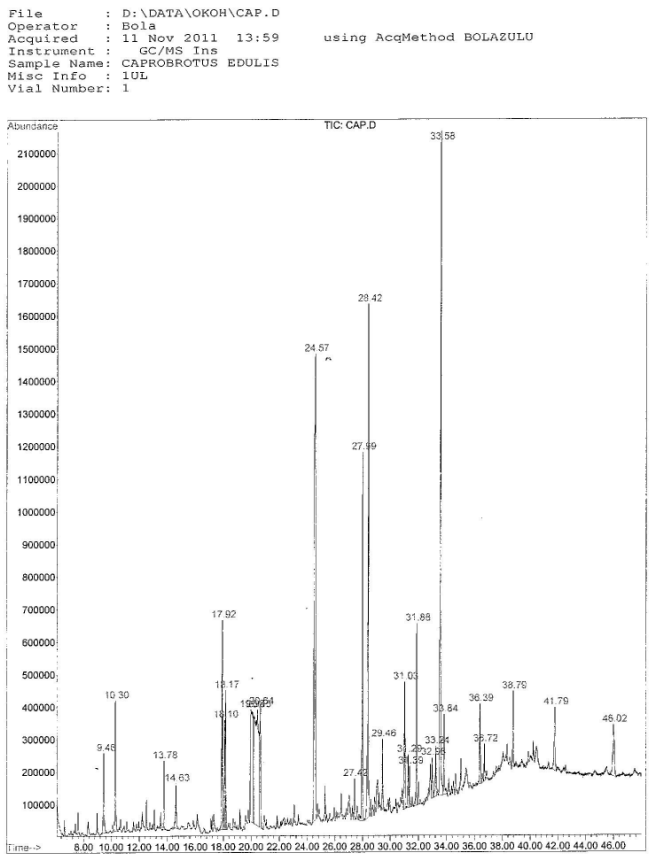

Figure 1:A typical Gas chromatography profile showing the chemical analysis of *M. edule*essential oil

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Statistical analysis

The experiments were done in triplicate and the data reported as the mean ± SD. Analysis of variance was performed by one way ANOVA using SPSS statistical software, version 20. A probability value at P <0.05 was considered statistically significant.

Results and Discussion

Chemical compounds of the essential oil

Hydro-distilled essential oil from fresh *M. edule* leaf analysed by GC-MS resulted in the identification of 28 compounds representing 99.99 % of the total oil. The oil was a pale yellowish liquid with a fine-agreeable characteristic aroma.

The major compounds of the essential oil, based on their mass spectra peak areas (Figure 1), were the Tetracosamethylcyclododecasiloxanes, which appeared 5 times with area peaks of 22.51%, followed by Tetradecamethylcycloheptasiloxane (appearing thrice) (23.76 %), Octadecane (appearing thrice) (2.56 %), Nephthalene (appearing twice) (3.93 %) and Eicosane (appearing twice) (4.0 %), (Table 1). These compounds are mainly used in the cosmetic industries to produce deodorants, sun blocks, hairsprays and skincare. They are also used as effective industrial cleaning agents and in dry-cleaning industries (Bogen et al., 2008).

Hundreds of phytoconstituents present in essential oil are differentiated by their terpene hydrocarbons. It is well documented that terpenes with different hydrocarbons are known to reduce accumulated toxins from the liver and kidneys (Torregrossa and Dearing, 2009). Bistrimethulesilyl N-acetyl, Isoterpinolene and Nephthalenes were identified as monoterpenes with different molecular weights contributing to 10.6 % peak area. Oxygenated monoterpene were found to be the highest (36.61 %) constituents in the crude extract. Essential oils containing monoterpene hydrocarbons have offered a variety of healing properties, especially their ability to restore or correct information in the DNA of a living cell and enhancement of other therapeutic components (Martins et al., 2012). Isoterpinolene, one of the major monoterpenes observed in the study was reportedto be capable of protecting human cells from free radical mediated oxidative stress (Santhi et al., 2012). It has been said that oxygenated monoterpene compounds are more valuable than the monoterpene hydrocarbons due to their contribution to the fragrance of the essential oil (Kamal et al., 2011; Martins et al., 2012). Hence the oxygenated compounds are highly odoriferous and most valuable.

The percentage amount of 3.58% Octadecanes, 1-octadecane and Nonadecane representing sesquiterpene hydrocarbons, were relatively very low compared to the oxygenated sesquiterpene, which had a peak area of 9.28%. Essential oil containing sesquiterpenes has been used to treat inflammatory and allergic infections (Carson et al., 2006; Ci et al., 2012). Research has found that people who consistently use essential oils that contain sesquiterpenes, have a higher level of resistance to illness than the average person. Further indications revealed that if such an individual eventually falls ill, he or she showed a 60-70 % faster recovery than those who did not use essential oils (Palombo, 2011; Astani et al., 2011).

Two Eicosanes with a total peak area of 1.43 % were the major concentrated diterpenes detected in the essential oil. Oxygenated diterpene constituents accounted for the third most concentrated hydrocarbons found in leaves, with a total oil content of 19.24 %. Of these, Phytol content gave the highest amount with a peak area of 12.41 %, followed by all the Tetradecamethylcycloheptasiloxanes, having a peak area of 6.83 %. The total amount of fatty acids and their methyl esters, present in the oil extract, were found to be 19.25 %. From Table 1 it is clear that benzoic acid represents the highest amount of fatty acids (15.68%) of the essential oil.

Several bioactive compounds have been isolated from *M. edule*, such as rutin, cactichin, ferulic acid hyperoside, oleanolic acid, catechin and epicatechin (Van der watt and Pretorius, 2001; Martins et al., 2010). Unfortunately, there is no available information on the chemical constituents from *M. edule* leaf essential oil. The different phytochemical constituents of monoterpene, sesquiterpenes, diterpenes and fatty acid esters have been reported to have antioxidant, antimicrobial and immune-modulating activities (Chokoe et al., 2008; Bouftira et al., 2009; Schelz et al., 2010). Martins *et al*. (2010) evaluated the mechanism of action of *M. edule* which had the potential of reversing the resistance of mouse lymphoma cells to chemotherapeutic agents.

Chemical compounds of the hexane, acetone and ethanol extracts

The identified phyto-constituents in the hexane, acetone and ethanol extracts from the leaves of *M. edule* are presented in Table 2, 3 and 4, respectively.

The scan spectrum of the GC-MS analysis identified 7 chemical compounds, with a probability percentage greater than 70%, in the hexane extract, which included 2-Pentadecanone,6,10,14-trimethyl, Phytol, n-Hexadecanoic acid, 4,8,12,16-Tetramethylheptadecan-4olide, Octadecanoic acid, α-Amyrin and Lupeol. The individual fragmentations of these compounds are illustrated in Figure 2 A-G, respectively. GC-MS analyses of the acetone extract, led to the identification of seventeen chemical compounds. The major phyto-constituents with probability percentage greater than 70%, were Phytol, n-hexadecanoic (dibutyl ester) acid, α-Amyrin and Lupeol (Figure 3 A-D). GC-MS chromatogram of the ethanol extract showed three distinct peaks of α-Amyrin, β-Amyrin, and Lupeol, but only α-Amyrin and Lupeol had a probability percentage greater than 70%. (Figure 4 A and B).

The various compounds observed from the three solvent extracts differed significantly from each other, although some of the phyto-constituents found in the hexane extract were also present in both the acetone and ethanol extracts. In terms of probability percentage (greater than 70%), 4,8,12,16-Tetramethylheptadecan-4-olide (86.9%), 2-Pentadecanone, 6,10,14-trimethyl (85.4%), n-Hexadecanoic acid (78.9%), Phytol (77.2%), α-Amyrin (72.2%) and Lupeol (71.3%), matched the mass spectra and retention indices as listed in the Wiley 275 library. These compounds have been found to have hypocholesterolemic activity, antioxidant and lubrication activity (Kumar et al., 2010). Anticancer and anti-proliferative activity were shown to be inhibited by tetradecanoic acid, α-Amyrin and Octadecahydro-2H-picen-3-one, while Phytol, Lupeol, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) ethyl palmitate and n-Hexadecanoic were reported to have antimicrobial, anti-inflammatory, antioxidant and hepatoprotective activity (Kumar et al., 2010).

Antifungal activity of the extracts

Microbiological screening of the essential oil as well as the four successive plant fractions, showed MIC activity against the fungal isolates, which was comparable to the standard antibiotics such as nystatin and amphotericin B drugs (Table 5).

In the present study, the essential oil tested was found more effective in reducing the organisms when compared with the four solvent extracts used (Table 5). In the same manner, the MIC activity of the essential oil was comparable with nystatin and amphotericin B which were used as controls. Essential oil from *M. edule* had MIC values from 0.02-0.08 mg/ml against four of the five isolates, with only *C. rugosa* requiring a significantly higher amount of 0.31 mg/ml. These values were in the same order as most of the MIC values for the control nystatin and

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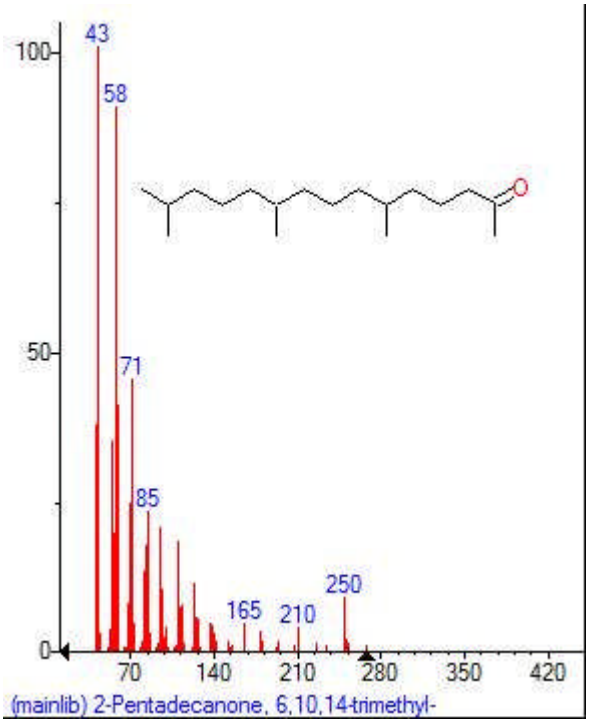
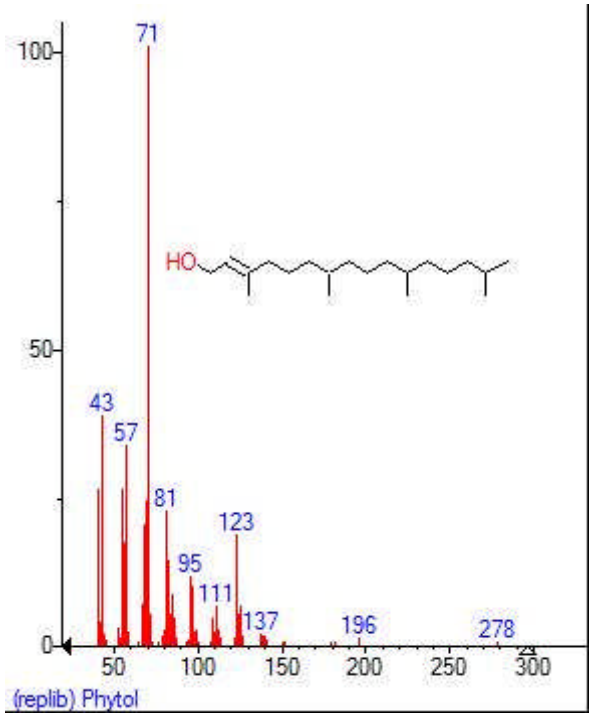
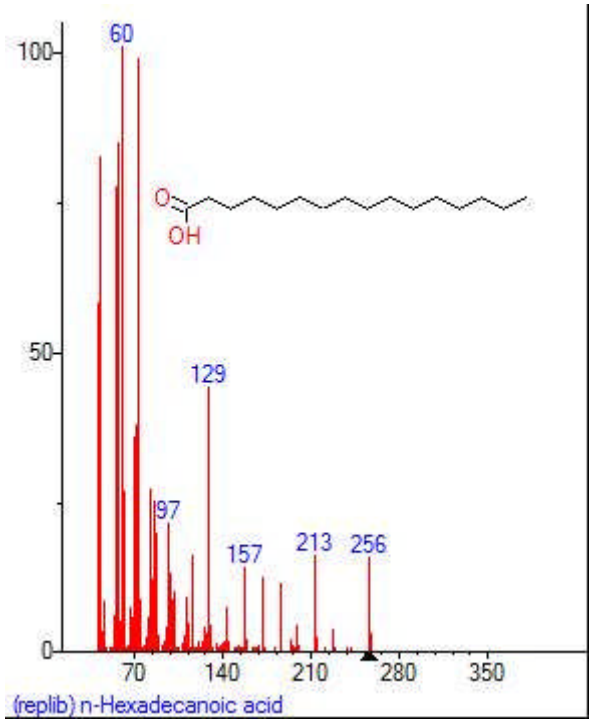


2A



2B



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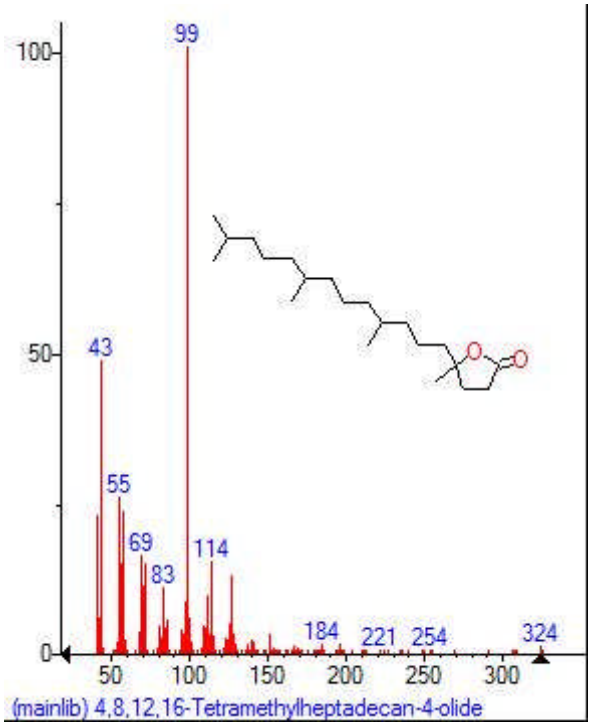

2D

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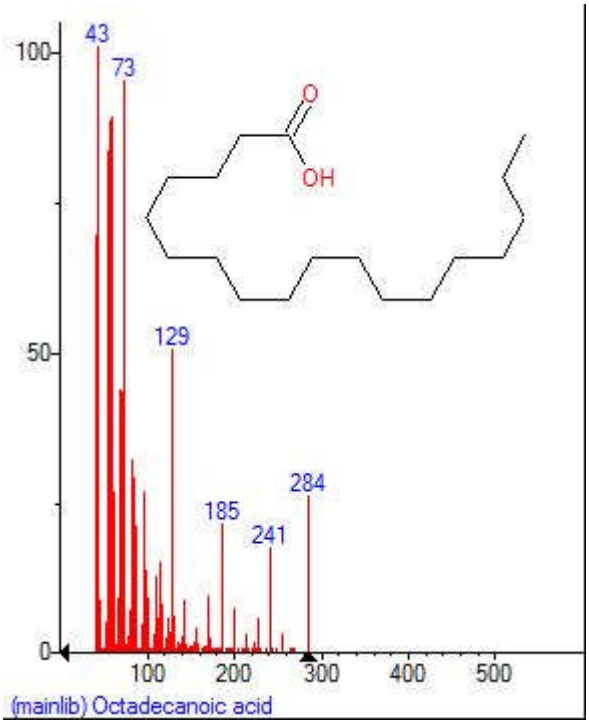

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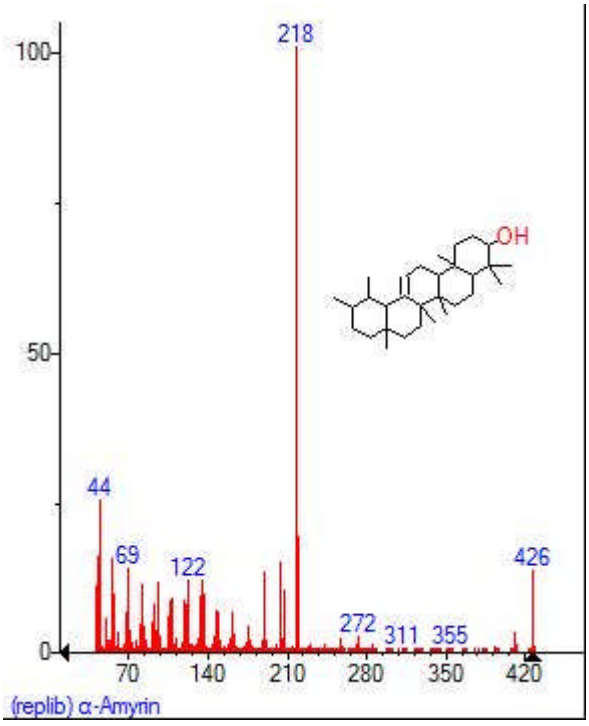
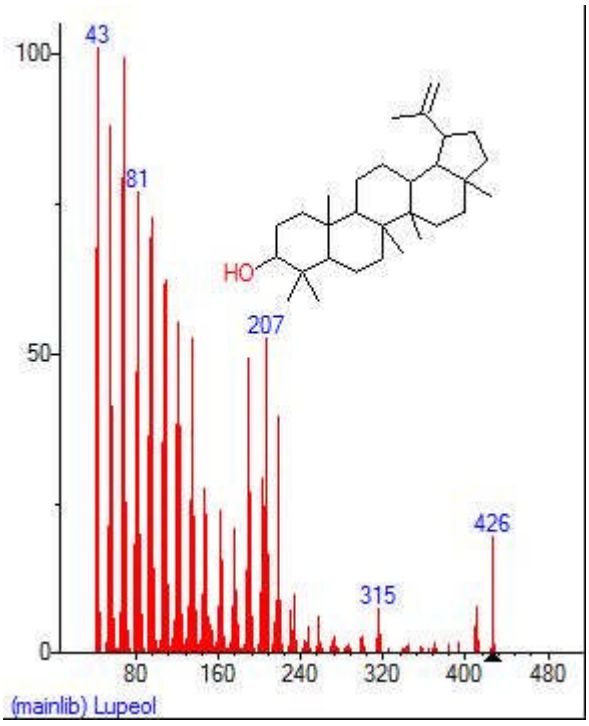


2F



2G

Figure 2: A-G: GC-MS peak view of the important components found in hexane extract. A: 2-Pentadecanone,6,10,14-trimethyl; B: Phytol; C: n-Hexadecanoic acid; D: 4,8,12,16-Tetramethylheptadecan-4olide; E: Octadecanoic acid; F: α-Amyrin and G: Lupeol.

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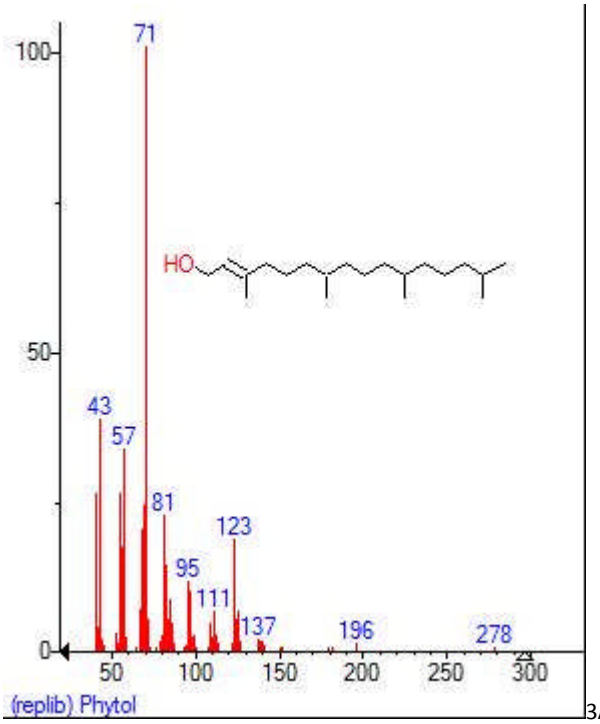
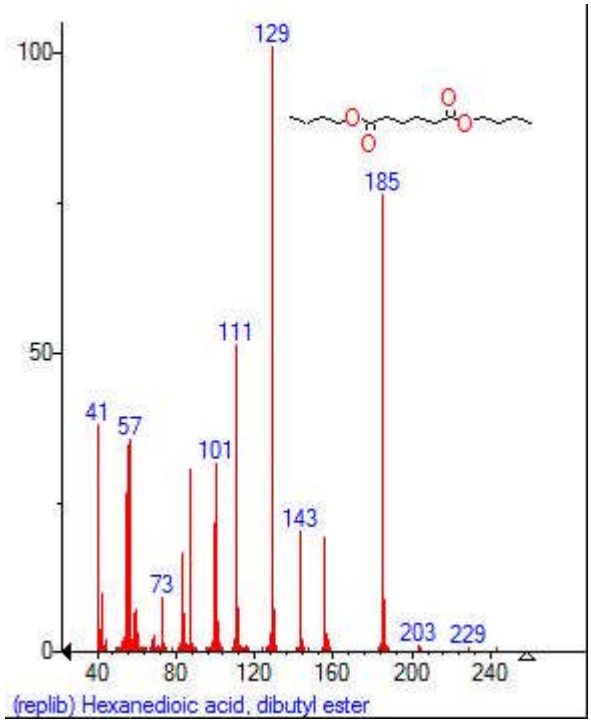


3A



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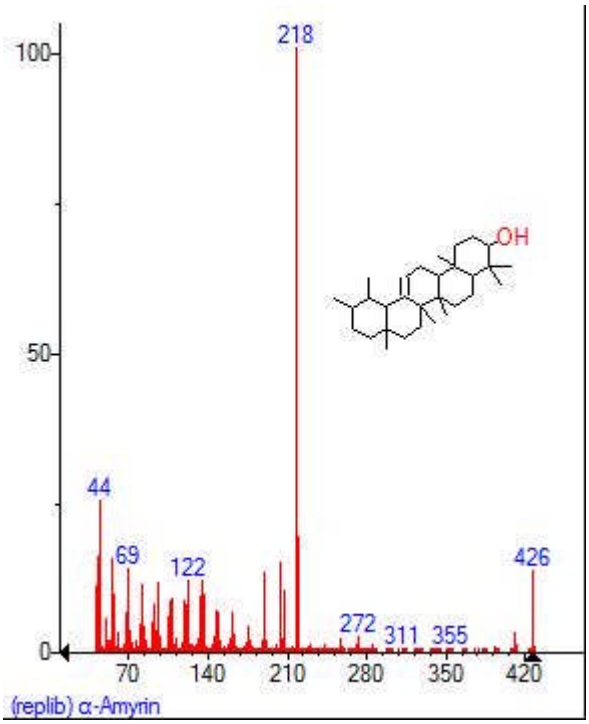

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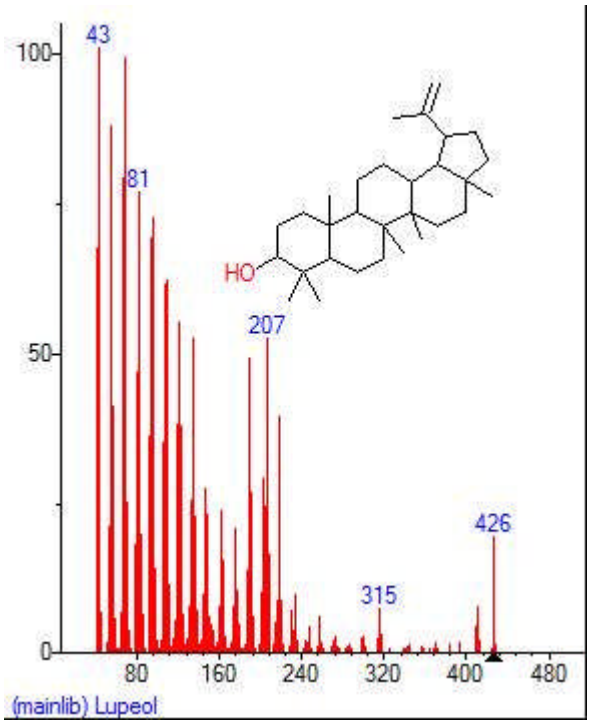

3D

Figure 3: A-D: GC-MS peak view of the important components found in the acetone extract. A: Phytol; B: Hexadecanoic (dibutyl ester) acid; C: α-Amyrin and D: Lupeol

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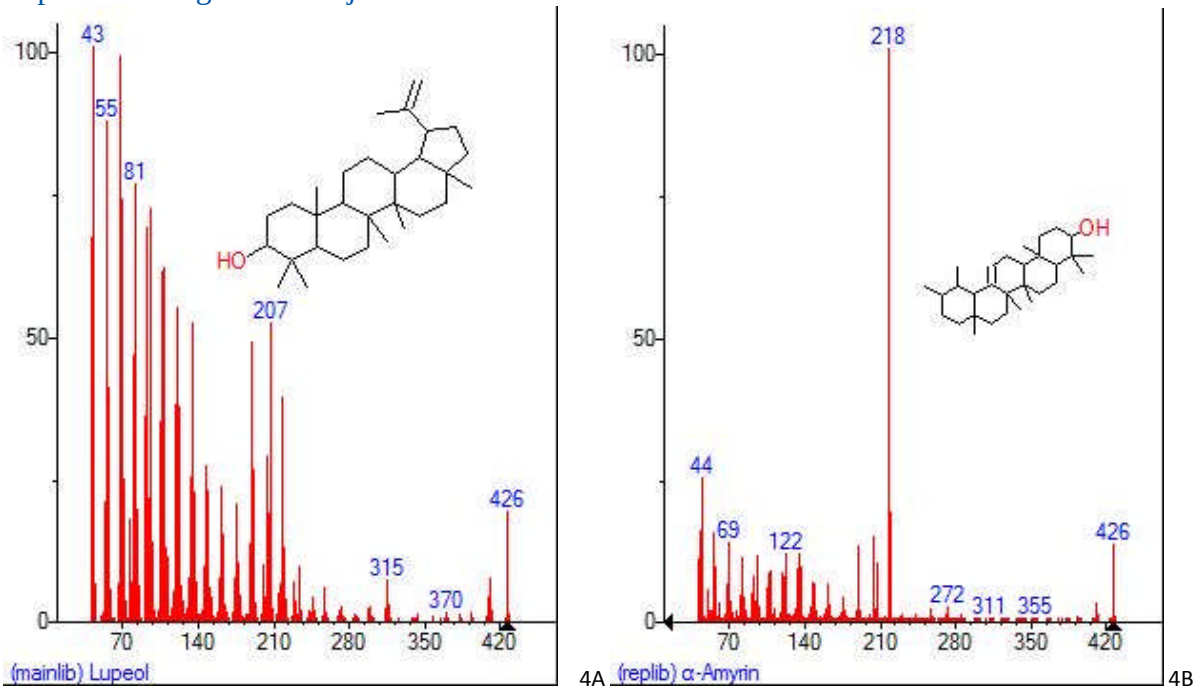

Figure 4: A and B: GC-MS peak view of the important components found in ethanol extract. A: Lupeol and B: α-Amyrin.

Table.1: Compounds obtained from GC/MS analysis of *M. edule* leaf part essential oil

Percentage composition of *M. edule*essential oil analysed by GC/MS				
PK/ No	Compounds	Kovats ndex	Peak %	Chemical formula
	Monoterpenes		**10.6**	
1	Isoterpinolene	1429	0.95	$C_{10}H_{16}$
2	Nephthalene, 1,2-dihydro-2,5,8-tri	1546	2.03	$C_{12}H_{10}$
3	Nephthalene, 1,2-dihydro-2,5,8-tri	1548	1.90	$C_{12}H_{10}$
4	Bistrimethylsilyl N-acetyl EICOSAS	1978	5.72	$C_{15}H_{33}NO_5Si_3$
	Oxygenated monoterpenes		**36.61**	
5	Mercaptoacetic acid, bis (trismethylsilyl)	3740	2.07	$C_8H_{20}O_2SSi_2$
6	Eicosamethylcyclodecasiloxane	1936	2.58	$C_8H_{24}O_4Si_4$
7	N-Octanol	1297	1.59	$C_8H_{18}O$
8	Nonylaldehyde	1345	2.29	$C_9H_{18}O$
9	Trans-β-demascenone	1538	3.42	$C_{13}H_{18}O$
10	Trans-2-tridecenal	1406	0.85	$C_{13}H_{24}O$
11	Tetradecamethylcycloheptasiloxane	1627	7.39	$C_{14}H_{42}O_7Si_7$
12	Tetradecamethylcycloheptasiloxane	1646	13.57	$C_{14}H_{42}O_7Si_7$
13	Tetradecamethylcycloheptasiloxane	1654	2.85	$C_{14}H_{42}O_7Si_7$
	Sesquiterpenes		**3.58**	
14	Octadecane	1991	0.64	$C_{18}H_{38}$
15	Octadecane	2092	1.12	$C_{18}H_{38}$
16	1-octadecene	2266	0.80	$C_{18}H_{36}$
17	Nonadecane	2284	1.02	$C_{18}H_{40}$
	Oxygenated sesquiterpene		**9.28**	
18	2-pentadecanone,6,10,14-trimethyl	2014	9.28	$C_{18}H_{36}O$
	Diterpenes		**1.43**	
19	Eicosane	2215	0.65	$C_{20}H_{42}$
20	Eicosane	2439	0.78	$C_{20}H_{42}$
	Oxygenated diterpenes		**19.24**	
21	Phytol (2-Hexadecen-1-o1, 3,7,11,15-tetramethyl)	2289	12.41	$C_{20}H_{40}O$
22	Trisiloxane,1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy] (Tetracosamethylcyclododecasiloxane)	2302	1.64	$C_{24}H_{72}O_{12}Si1_2$
23	Tetrasiloxane,1,1,1,5,7,7,7-heptamethyl-3,bis[(trimethylsilyl)oxy] (Tetracosamethylcyclododecasiloxane)	2420	1.66	$2C_{24}H_{72}O_{12}Si1_2$
24	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tri(trismethylsiloxy) tetrasiloxane (Tetracosamethylcyclododecasiloxane)	2538	1.69	$2C_{24}H_{72}O_{12}Si1_2$
25	Tetrasiloxane-1,1,1,5,7,7,7-heptamethyl-3,3 bis[(trismethylsilyl)oxy)] (Tetracosamethylcyclododecasiloxane)	2680	1.84	$2C_{24}H_{72}O_{12}Si1_2$
	Fatty acids		**19.25**	
26	Benzoic acid, 2,5-bis (trimethylsiloxy-,trimethylsilyl ester (Tetracosamethylcyclododecasiloxane)	1841	15.68	$C_{16}H_{30}O_4Si_3$
27	Hexadecanoic acid, ethyl ester	2183	0.89	$C_{18}H_{36}O_2$
28	Hexadecanoic acid, 1-methylethyle ester	2215	2.68	$C_{19}H_{38}O_2$
	Total compounds (%)		**99.99**	

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Table 2: Phyto-constituents identified in the hexane extract of *M. edule*

	RT	Compounds	Formula	Prob (%)
1	3.9	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	85.4
2	4.51	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	26.6
3	5	Heptacosane	$C_{27}H_{56}$	20.1
4	5.3	1-Heptatriacotanol	$C_{37}H_{76}O$	16.3
5	5.54	n-Octyl-5-oxoheptadecanamide	$C_{25}H_{49}NO_2$	32.4
6	5.75	Heptacosane	$C_{27}H_{56}$	15.6
7	6.09	Dodecanoic acid	$C_{12}H_{24}O_2$	45
8	6.732	Heptacosane	$C_{27}H_{56}$	19.6
9	7.78	Phytol	$C_{20}H_{40}O$	77.2
10	7.97	Heptacosane	$C_{27}H_{56}$	16.4
11	8.6	Dibutyl phthalate	$C_{16}H_{22}O_4$	11
12	8.67	n-Hexadecanoic acid	$C_{14}H_{28}O_2$	74.6
13	8.93	2-Tertbutyl cyclohexylpropylphosphonofluoridate	$C_{13}H_{26}FO_2P$	10.7
14	9.572	Heptacosane	$C_{27}H_{56}$	29.2
15	10.55	2-Pyrrolidinone, 1-(9-octadecenyl)	$C_{22}H_{41}NO$	15
16	11.47	Pyrrolidine, 1-(1-oxo-7,10-hexadecadienyl)	$C_{20}H_{35}NO$	39.8
17	12.86	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	78.9
18	13.96	Nonacosane	$C_{29}H_{60}$	23.4
19	14.331	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	86.9
20	18.09	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl	$C_{30}H_{50}$	49.7
21	19	Octadecanoic acid	$C_{18}H_{36}O_2$	69.3
22	19.87	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	19.7
23	8.6	Tetradecanoic acid	$C_{14}H_{28}O_2$	11
24	20.6	Tetratriacontane	$C_{34}H_{70}$	11.1
25	21.62	9,12-Octadecadienoic acid (Z,Z)-2 3-dihydroxypropyl ester	$C_{18}H_{32}O2$	40.9
26	24.07	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$	20.2
27	27.08	Eicosanoic acid	$C_{20}H_{40}O_2$	56.2
28	28.83	Nonacosane	$C_{29}H_{60}$	9.95
29	32.4	α-Amyrin	$C_{30}H_{50}O$	26
30	33.97	9,19-Cyclolanostan-24-en-3-ol, acetate, (3β)	$C_{32}H_{52}O_2$	20.6
31	34.87	1-Heptatriacotanol	$C_{37}H_{76}O$	22.2

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32	40.19	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)	$C_{32}H_{52}O_2$	28.9
33	40.57	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)	$C_{32}H_{52}O_2$	35.9
34	43.5	α-Amyrin	$C_{30}H_{50}O$	71.9
35	48.09	Lupeol	$C_{30}H_{50}O$	71.3
36	56.05	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	$C_{27}H_{46}O$	37.2
37	57.35	Vitamin E	$C_{29}H_{50}O_2$	48.2
38	58.06	17-(1,5-Dimethylhexyl)-2,3-dihydroxy-10,13-dimethyl-1,2,3,7,8,9,10,11,12,13,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-6-one	$C_{27}H_{44}O_3$	26
39	59.83	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	63.5

Table 3: Phyto-components identified in the acetone extract of *M. edule*

	RT	Compounds	Formula	Prob (%)
1	4.51	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	26.6
2	5.113	6,6-Dimethyl-10-methylene-1-oxa-spirodecane	$C_{12}H_{20}O$	28.2
3	5.3	1-Heptatriacotanol	$C_{37}H_{76}O$	16.3
4	6.09	Dodecanoic acid	$C_{12}H_{24}O_2$	45
5	6.286	17-Pentatriacontene	$C_{35}H_{70}$	17.9
6	7.367	17-Pentatriacontene	$C_{35}H_{70}$	18.6
7	7.78	Phytol	$C_{20}H_{40}O$	77.2
8	8.6	Tetradecanoic acid	$C_{14}H_{28}O_2$	11
9	8.67	n-Hexadecanoic acid (dibutyl ester)	$C_{14}H_{28}O_2$	74.6
10	9.572	Heptacosane	$C_{27}H_{56}$	29.2
11	12.86	n-Hexadecanoic acid (bis-2-ethylhexyl ester)	$C_{16}H_{32}O_2$	78.9
12	13.96	Nonacosane	$C_{29}H_{60}$	23.4
13	42.918	α-Amyrin	$C_{30}H_{50}O$	70.9
14	43.5	α-Amyrin	$C_{30}H_{50}O$	71.9
15	43.918	α-Amyrin	$C_{30}H_{50}O$	72.2
16	48.09	Lupeol	$C_{30}H_{50}O$	71.3
17	48.89	Lupeol	$C_{30}H_{50}O$	79.5

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Table 4: Phyto-constituents found in the ethanol extract of *M. edule*

	RT	Compounds	Formula	Prob (%)
1	32.4	β-Amyrin	$C_{30}H_{50}O$	26
2	42.918	α-Amyrin	$C_{30}H_{50}O$	70.9
3	48.09	Lupeol	$C_{30}H_{50}O$	71.3

Table 5: Minimum inhibitory concentration (MIC) of the various extracts of *M. edule* against the five fungi, *C. albican*, *C. krusei*, *C. rugosa*, *C. glabrata* and *C. neoformans.*

	Minimum inhibitory concentration						
Test organisms	Hexane (mg/ml)	Acetone (mg/ml)	Ethanol (mg/ml)	Water (mg/ml)	Essential oil (mg/ml)	Nystatin (mg/ml)	Amphotericin B (mg/ml)
C. albican	0.02 [a]	0.63[b]	1.25[b]	-	0.02 [a]	0.02 [a]	0.02 [a]
C. krusei	0.02 [a]	0.04 [a]	1.25[b]	-	0.04 [a]	0.02 [a]	0.009 [a]
C. rugosa	1.25[b]	0.16[b]	-	-	0.08 [a]	0.02 [a]	0.009 [a]
C. glabrata	0.08 [a]	1.25[b]	-	-	0.31[b]	0.04 [a]	0.02 [a]
C. neoformans	0.31[b]	1.25[b]	-	-	0.08[a]	0.009 [a]	0.02 [a]

** Values within a column followed by the different superscript are significantly different ($P<0.05$). The minus sign (-) indicates a negative result

amphotericin B. Only nystatin against *C. neoformas* and amphotericin B against *C. krusei* and *C. rugosa* had lower MIC value of 0.009 mg/ml. The *in vitro* antifungal activity of the essential oil of *O. grastissimum* demonstrated fungicidal activity against all the *Candida* species studied (Abad et al., 2007). The MIC values for the acetone and ethanol extracts were generally higher than those found for the essential oil. The MIC of hexane extract gave the best activity against *C. albican* (0.02 mg/ml), *C. krusei* (0.02 mg/ml) and *C. glabrata* (0.08 mg/ml). *Candida rugosa* and *C. neoformans* showed inhibitory activities at 1.25 mg/ml and 0.31 mg/ml. Steenkamp et al. (2007) reported similar hexane extract activity against all the fungal strains used in their study. Water extracts were not able to control the growth of the five fungi, even at the highest concentration of 5mg/ml. This result agrees with the study done by Rasool et al. (2008), who reported that water extract treatment from *Coriolus versicolor* and *Rauvolfia tetraphylla* was ineffective against fungal organisms. Thus our results revealed that essential oil and hexane extracted samples were more effective in controlling the growth of fungal infections. These results agree with those reported by Steenkamp et al. (2007) and Otang et al. (2011).

Conclusion

The results obtained from the GC-MS resulted in the identification of 28 hydrocarbons of the total essential oil. A total of fifty-nine compounds were observed from the hexane, acetone and ethanol extracts. The Phytoconstituents present in the essential oil are from the families of monoterpenes, sesquiterpenes, diterpenes, and fatty acids esters (Cowan, 1999). These terpenoids are reported to have a wide range of biological activities against cancer, malaria, inflammation and a variety of infectious diseases (Cowan, 1999; Chen et al., 2011). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Newman and Cragg, 2007; Rattan, 2010). Oxygenated monoterpenes occupy the major constituents of the oil, followed by fatty acids and oxygenated diterpenes. Oxygenated monoterpenes have been reported specifically to have anti-inflammatory, antiseptic, antibacterial and antiviral properties (Carson et al., 2006; Chen et al., 2011). The therapeutic potency of *M. edule* used as traditional medicine thus contains properties that inhibit the growth of fungi, viruses and other microbes (Chen et al., 2011). Conversely, those extracts that produced weak or no inhibitory results such as water and ethanol extracts do not mean absence of bioactive constituents nor that the plant is inactive, since plant extracts have been reported to act in other ways such as by stimulating the immune system of the patient or by creating internal conditions that are unfavourable for the multiplication of the microorganism (Buwa and Afolayan, 2009). According to our survey, water and alcohol extracted plant samples are the most common medications used by traditional healers to treat the common ailments mentioned above. However, the dosages prescribed by these herbalists are higher than the highest concentration of 5 mg/ml used in this study. For instance three to four cupfuls per day is given to adults and one teaspoon 3 times daily for children. This observation supported the reports of Steenkamp et al. (2007) and Otang et al. (2011), who revealed thatwater extracts tested against fungal strains did not give any activity, probably due to its polarity or the low the dosage used.

Mesembryanthemum edule has been confirmed as a candidate species for future studies as a source of novel and alternative remedies for the treatment of microorganism infections. Further studies also need to be conducted to verify the efficacy of water and ethanol extracts as potential treatments to manage HIV infections and possible sources of future drugs in the treatment of AIDS.

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