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Solanum aculeastrum Dunal berries: Phytochemical profiling and GC-MS analysis of methanolic extract and n-hexane, dichloromethane, ethyl acetate and n-butanol fractions

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Abstract

Background: *Solanum aculeastrum* is reportedly used in several diseases including gonorrhea, bronchitis, jigger infestations and wounds, and cancers. We conducted an exhaustive phytochemical and GC-MS profiling of its methanol extract and solvents' fractions.

Methods: About 4500g of dried berries of *S. aculeastrum* was extracted with methanol, part of which was partitioned into fractions of n-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous fractions. These fractional preparations were subjected to phytochemical and GC-MS profiling except aqueous fraction.

Results: The percentage yield of 430.69g of methanol extract of berries of *S.aculeastrum* was 9.57%w/w, while the yields of dried 19.4g of n-hexane, 38.4g of DCM, 6.4g of ethyl acetate, 81.03g of n-butanol and 21.2g of the freeze-dried aqueous fractions were 7.08%w/w, 14.03 %w/w, 2.33%w/w, 29.60%w/w and 7.74%w/w respectively. The relative presence of glycosides, alkaloids, tannins, flavonoids, terpenoids, phenols, saponins were confirmed except quinones. GC-MS profiling of methanol extract identified 32 compounds including alkane, alcohol, carbohydrate, fatty acids, terpenoids, glycerides, vitamins and some unclassified compounds. 25 compounds including terpenoids, alkene, fatty acids, vitamin and unclassified compounds were identified in n-hexane fractions. The DCM fraction yielded 20 compounds including isoprenoid, terpenoids, amino acids, carboxylic acid and unclassified compounds. 22 compounds were identified in ethyl acetate fraction including phenol, fatty acids, alcohol, terpenoids, glycerolipid and unclassified compounds. The n-butanol fraction yielded 11 compounds including fluorinated aromatic substance, hormonal antineoplastic and a non-steroidal anti-inflammatory agent.

Conclusion: This study has further elaborated on the bioactive compounds in berries of *S. aculeastrum*, to aid robust understanding of its pharmacological activities.

Keywords: Phytochemical; GC-MS; Methanol; n-Hexane; Dichloromethane; Ethyl acetate; n-Butanol

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1. Introduction

The systematic recording, analysis and interpretation of traditional medicine services has been supported by the International Classification of Disease (ICD-11) [1], implying the official recognition of the use of traditional medicine in management of disease conditions globally. Over the years, traditional medicine has been in use in the treatment of many diseases across the globe, as about 80% of the global populace depend on it, and plant-based therapies are known to be the most popularly used alternative/complementary remedies to primary health-related challenges [2], [3]

Natural products have over the years remained a reservoir for identification of important bioactive compounds for combating a range of health-related challenges. Herbs have been used in treatment of different diseases and improvement of health for many years [4]. During the past decades, global attention has been gained by herbs or their extracts as a result of their significant efficacy in treating and preventing some diseases [5]. Many countries in Africa still rely partly on traditional medicine as about 70–95% populations of developing countries use herbal drugs for basic health care [6]. Furthermore, many Kenyans give credence to the potency of herbal medicine, even when they have access to conventional medicine. In some cases, they combine both herbal and orthodox medicine, especially if they are afflicted with chronic ailments such as HIV/AIDS, hypertension, infertility, cancer and diabetes [7].

Plants possess different phytochemicals also referred to as secondary metabolites which differ in terms of their quantities and nature [8]. The berries, leaves, stems, roots, barks and flowers contain various phytochemicals such as anthraquinones, saponins, tannins, flavonoids, terpenoids, alkaloids and phenols which possess medicinal properties including anti-diarrhea, anti-inflammatory, antimycobacterial, antioxidant, antiplasmodial, anticancer and anti-ulcer activities [9].

Solanum aculeastrum Dunal is a shrub belonging to the family Solanaceae whose species name, *aculeastrum*, signifies the thorns that beautifies most parts of the shrub. It is commonly known as Ochok in the Western part of Kenya and referred to as Omotobo by the Abagusii community in Nyamira County of Kenya. Other names of the plant are soda apple or goat bitter apple [10]. It is a plant whose origin is traced from the tropical Africa to South Africa in different climatic conditions and found mostly in woodland, grassland, forest margins and different soil types including reddish brown clay-loam and brown sandy loam as well as sandy soils [11], [12].

The Abagusii community of Nyamira County in Kenya uses the fruits and leaves of *S. aculeastrum* Dunal in different forms for the management of swollen joints in fingers, gangrene, gonorrhea, bronchitis, jigger infestations and wounds (*Tungiasis*), toothaches, rheumatism and in ringworm in cattle, and as eyewash. Also, preparation of the root bark as a decoction is used in the country for treatment of acne and for sexually transmitted bacterial diseases such as gonorrhea [13]. The plant parts such as the leaves, root bark, and fruits have been used to manage various ailments including skin and cervical cancer [14]. The leaves and fruits are used orally in management of digestion and stomach disorders, gonorrhea, and cancer [11], [15]. The berries of *Solanum aculeastrum* Dunal and leaf materials demonstrated potentials for anti-leishmanial activities [10]. Also, methanol and aqueous extracts of the berries have been shown to have moderate antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria [16].

The bioactive compounds such as solanculine A, *β*.-solanmarine isolated from the root bark and solanmargine isolated from the berries possess activities against molluscicidal infections, with the steroidal alkaloid glycosides and saponins suggested to be responsible for these activities [17]. The crude extract and aqueous fraction of the fruits of *S. aculeastrum* have also been reported to possess potent non-selective cytotoxicity and inhibition of P-glycoprotein against various cancer types, which was linked to the steroidal alkaloid solanmargine [18]

Consequent to the persistent interest in traditional medicine over the years, with phytotherapy becoming more popular amongst patients, the need for a continuous scrutiny of potential medicinal plants including screening for their phytochemical and bioactive constituents remains paramount. It is in the light of the foregoing that we conducted this study to determine the secondary metabolites and bioactive constituents of methanol extract and n-hexane, ethyl acetate, dichloromethane, n-butanol, and aqueous fractions of the berries of *Solanum aculeastrum* Dunal through qualitative phytochemical screening and GC-MS analysis.

2. Materials and Method

2.1. Collection and Identification of Plant Materials

The fresh berries of *Solanum aculeasrtum* Dunal were sourced for and collected from homesteads from Kakamega County since it is not an endangered species and required no authorization before collection. The collected berries of *S. aculeasrtum* Dunal was identified by a taxonomist from the National Herbarium of Kenya, Nairobi, Kenya, where voucher specimen was deposited with voucher number allocated (PENDER/001).

2.2. Preparation of Plant Materials, Procedure for Whole Extraction and Fractionation

The collected berries of *S.aculeastrum* Dunal were cleaned to remove debris, shade-dried at ambient temperature for three weeks, with constant monitoring and repeated turning of berries to prevent contamination due to fungi. The dried berries of *S. aculeastrum* Dunal was grinded into powdered forms with the aid of an electric pulverizer at 8000RPM (Christy and Norris Ltd; Serial No: 51474) at the Chemistry Department of Kenyan Medical Research Institute (KEMRI), Nairobi, Kenya. A total of 4.5kg (4500g) of the blended *S. aculeastrum* Dunal berries was obtained which was macerated using 15litres of methanol being a strong polar solvent for 24hours and re-soaked three times for exhaustive extraction [19]. Whatman filter paper (No.1, 240mm) was used to filter the macerate and get rid of residues. The filtrate solution was concentrated *in Vacuo* using Rotary evaporator (Buchi R-300, Switzerland) under controlled vacuum pressure of 337 Mbar at 40°C [20].

The total amount of methanol crude extract of *S. aculeastrum* Dunal was 430.69g, from which 157.0g were set aside as total methanol extracts and the remaining 273.69g extracts proceeded to solvent-solvent fractionation extraction as per Kupchan modified scheme [21], [22]. Briefly, 273.69g Methanol crude extract was dissolved in 400 mL methanol/water (9:1 v/v) for n-hexane; (6:4 v/v) DCM; (4:6 v/v) ethyl acetate; (1:9 v/v) n-butanol; and sonicated for 30minutes for adequate mixing. Using 4 identical 500ml separating funnels,100 mL of the obtained solution was transferred to each funnel and partitioned with 200ml (1:2) of each of N-Hexane, Dichloromethane, Ethyl acetate, N-Butanol and water solvents in increasing order of polarity. Each partition was done for three times to ensure complete extraction of the compounds with respect to their various solvents and their polarities. The final aqueous fraction after n-butanol portioning was subjected to freeze-drying (Edwards, KA00000627, UK) to obtain aqueous fraction of the berries of *S. aculeastrum* Dunal.

The fractions of N-Hexane, Dichloromethane (DCM), Ethyl acetate, and N-Butanol were concentrated in vacuo with aid of rotary evaporator (Buchi R-300, Switzerland) at 35°C, 40°C, 45°C, and 45°C all at reduced pressure respectively. The dried n-hexane, DCM, ethyl acetate, and n-butanol resulted to 19.4g, 38.4g, 6.4g, and 81.03g respectively and the freeze-dried aqueous fraction gave 21.2g [23]. Thereafter, all dried extracts and fractions were kept in airtight containers at 4°C ready for use. The percentage yields of the whole methanol extracts and n-hexane, ethyl acetate, n-butanol, dichloromethane and aqueous fractions were then calculated using the formula below:

$$\text{Percentage yield of extracts} = \frac{\text{Weight of the obtained extract material}}{\text{Weight of original fine plant powder used}} \times 100$$

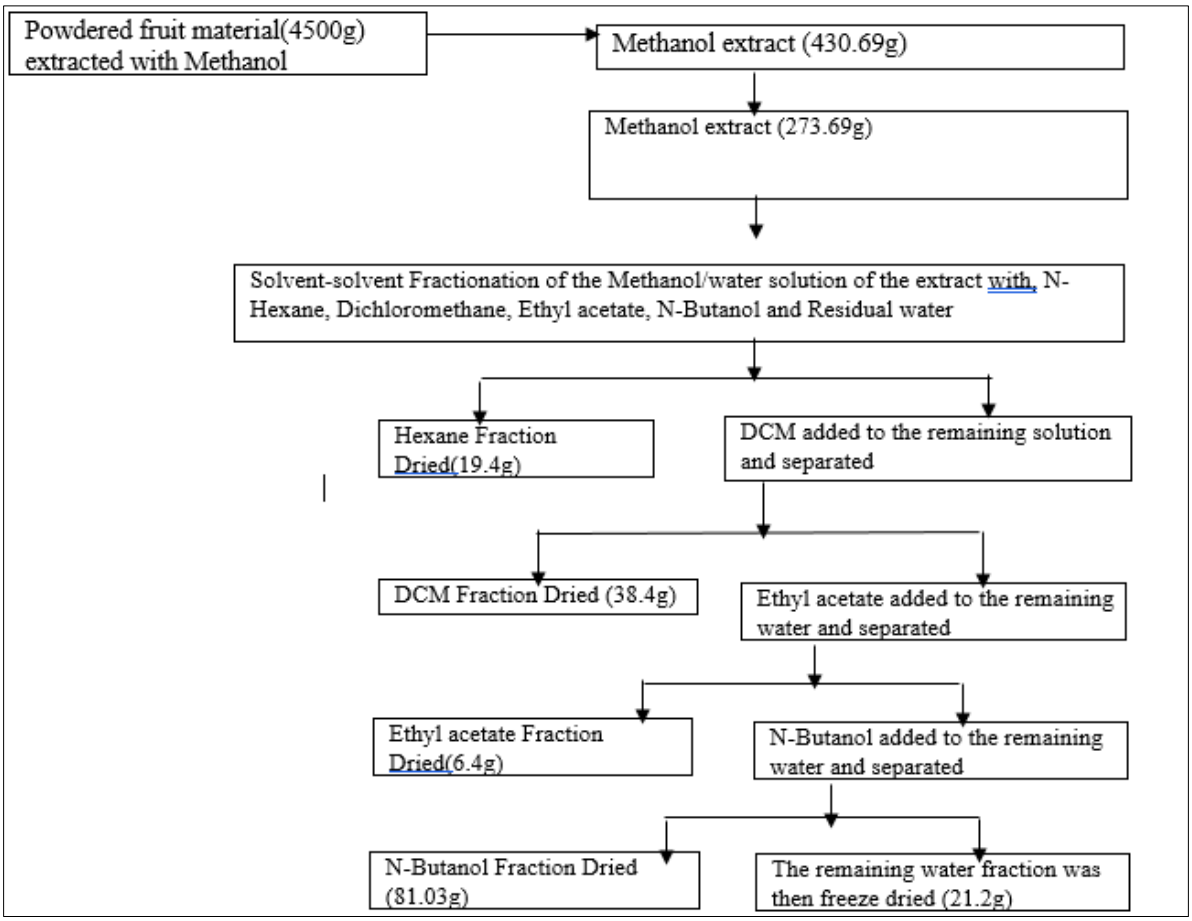


Figure 1 Protocol used for the solvent-solvent fractionation of the components in *Solanum aculeastrum* Dunal berries [21]

2.3. Qualitative Phytochemical Analysis of Plant Materials

Qualitative Phytochemical analysis for identification of plant secondary metabolites was done for whole methanol extract and n-hexane, ethyl acetate, dichloromethane, n-butanol fractions and residual aqueous extracts of *S. aculeastrum* Dunal berries using standard procedures [24]. 0.5g of each extract was dissolved in 50 mL of respective solvents of extraction. Reagents used were prepared freshly, ready for use. [25].

Table 1 Qualitative Phytochemical Analysis of whole Methanol extract and solvent fractions of berries of *S. aculeastrum* Dunal

Phytochemical	Test	Procedure	Observation
Alkaloids	Dragendroff's Test	I ml of filtrate + 2ml Dragendroff's reagent	A reddish-brown precipitate
Glycosides	Keller-Killiani's Test	1ml filtrate+1.5 ml glacial acetic acid+ 1 drop of 5% ferric chloride+conc H_2SO_4	A blue/green colored solution (in acetic acid layer)
Flavonoids	Alkaline reagent Test	2-3 drops of NaOH sol+ 2ml of filtrate	A deep yellow color, gradually disappears on addition of 5% HCL
Phenol	Ferric Chloride test	2ml of filtrate +aqueous 5% ferric chloride	Dark Green/bluish black color
Tannins	Braymer's test	2ml of plant extract+ methanolic sol of 10% ferric chloride	Formation of blue/greenish solution

Steroids	Salkowski test	2 ml of filtrate+2ml of chloroform + 10 drops of acetic anhydride and 2 drops of conc H_2SO_4 (Shake well and allow to stand)	Dark pink/red colour in lower chloroform layer
Quinones	Conc HCl test	2ml of filtrate + conc. (98%) conc HCl	Green color
Terpenoids'	Salkowski test	1ml of chloroform +2ml filtrate+1ml conc H_2SO_4	Reddish brown coloration of the interface
Saponins	Froth test	2 ml filtract+6ml water, shaken vigorously	Foam formation

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis to determine the composition of the chemical components of berries of *S. aculeastrum* Dunal whole methanol extracts and n-hexane, ethyl acetate, n-butanol, dichloromethane and aqueous extracts was done with the aid of a GC-MS system (Model; Shimadzu, GC-MS QP-2010SE) which had a low polarity BPX5 capillary column (30 m × 0.25 mm × 0.25 µm film thickness) with temperature of oven set at 55°C, consistently for 1min and increased by 10°C gradually after each minute until isothermal temperature of 280°C was achieved at 15 min 30 s being the final hold time. The carrier gas was helium (He) maintained at a constant flow rate of 1.08 ml / min and temperature of 200 °C was maintained as the injector temperature. Four (4) mins served as the solvent delay time and the diluted samples of 1µl of each solvent extract were injected automatically with the aid of an AS3000 autosampler coupled with GC in the split mode, split ratio (10:1). 200 and 250°C were set as the ion source and interface temperatures respectively. The EI mass spectra were collected at 70 eV in full scan mode over the range of m/z 35 to 550. Thereafter, qualitative identification of compounds detected in the extract was done with the aid of NIST mass spectra database.

3. Results

3.1. Plant Extraction and Phytochemical Screening

Table 2 Percentage yields of methanol extract, solvents fractions and residual aqueous extract of berries of *S. aculeastrum* Dunal

Solvents	Amount of dried extract (g)	Percentage yield (%w/w)
Methanol	430.69	9.57
N-Hexane	19.4	7.08
Dichloromethane	38.4	14.03
Ethyl acetate	6.4	2.33
N-Butanol	81.03	29.60
Aqueous	21.2	7.74

Table 3 Phytochemical constituents of methanol extract, solvent fractions and residual aqueous extract of berries of *S. aculeastrum* Dunal

	Crude	N-Hexane	DCM	Ethyl acetate	N-Butanol	Aqueous
Alkaloid	+++	++	+++	++	+++	+++
Glycosides	+++	+++	+++	++	++	-
Flavonoids	+++	-	++	++	++	+++
Phenols	+++	-	-	-	++	+++
Tannins	+++	-	+++	+	++	-

Steroids	+++	++	-	-	++	++
Terpenoids	+++	+	++	++	+++	+++
Quinones	-	-	-	-	-	-
Saponins	+++	-	-	-	-	+++

Key: + Present but mild: ++ Moderately present: +++ Highly present; - Absent

3.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

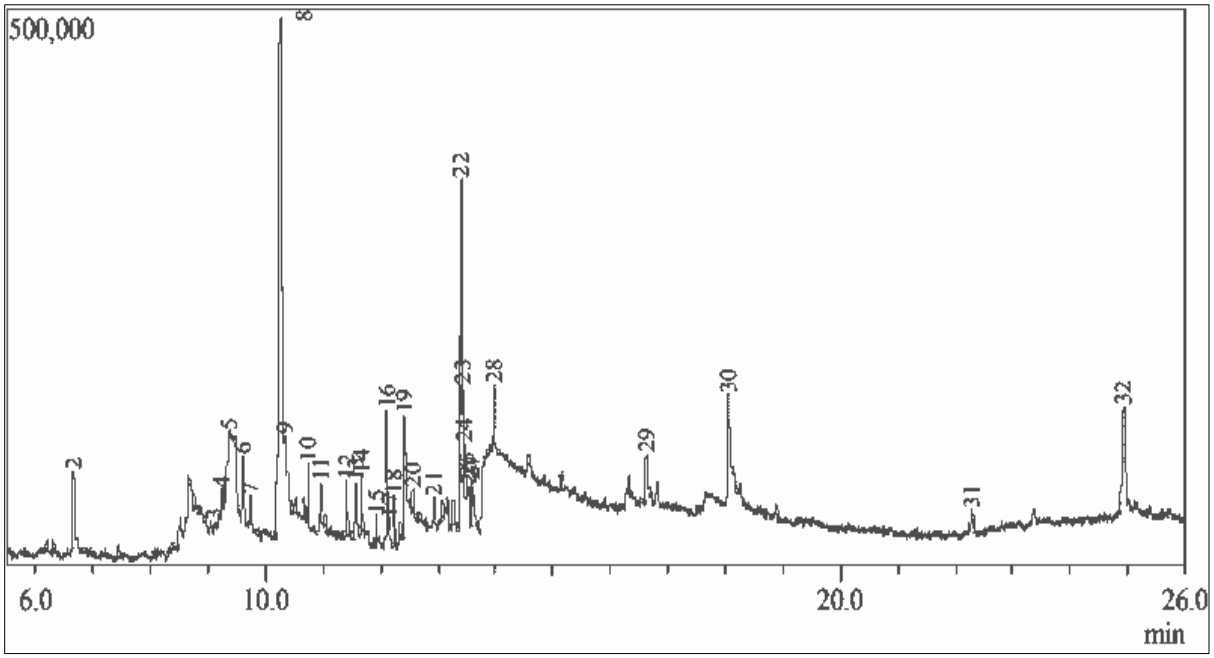


Figure 2 Chromatogram of the bioactive compounds in the methanol whole extract of *S. aculeastrum* Dunal berries

Table 4 The bioactive compounds present in methanol whole extract of *S. aculeastrum* Dunal berries identified by GC-MS

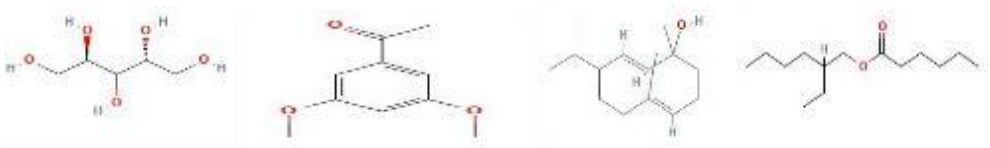
Peak No	Retention Time (min)	Identity of compound	Peak Area (%)	Molecular Weight (g/mol)	Molecular Formula	Class
1	5.067	Undecane	0.77	156.31	$C_{11}H_{24}$	Alkanes
2	6.653	2-Pentanol	2.23	88.15	C_5H1_{20}	Fatty Alcohols
3	9.105	Eicosapentaenoic Acid	1.22	302.5	$C_{20}H_{30}O_2$	Fatty Acids
4	9.233	Shyobunol	1.17	222.37	$C_{15}H_{26}O$	Terpenoid
5	9.369	D-Arabinitol	15.71	152.15	$C_5H_{12}O_5$	Carbohydrates
6	9.603	3',5'-Dimethoxyacetophenone	2.44	180.2	$C_{10}H_{12}O_3$	NC
7	9.742	Germacrene D-4-ol	1.11	222.37	$C_{15}H_{26}O$	Alcohol
8	10.252	Hexanoic acid, 2-ethylhexyl ester	29.98	228.37	$C_{14}H_{28}O_2$	NC
9	10.34	2,3-Dehydro-4-oxo-7,8-dihydro-.beta.-ionone	2.46	206.28	$C_{13}H_{18}O_2$	NC
10	10.739	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-,[1R(1.alpha.,2.beta.,3.alpha.,6.alpha.)]-	0.99	NMW	NMF	NC

11	10.96	9-Oxabicyclo[3.3.1]nonan-2-one,6-hydroxy-	1.4	156.18	$C_8H_{12}O_3$	NC
12	11.409	2,5,9-Tetradecatriene, 3,12-diethyl-	1.06	248.4	$C_{18}H_{32}$	NC
13	11.566	Solavetivone	1.15	218.33	$C_{15}H_{220}$	Terpenes
14	11.667	Globulol	1.36	222.37	$C_{15}H_{260}$	Terpenoid
15	11.929	2-(1,4,4-Trimethyl-cyclohex-2-enyl)-ethanol	0.48	168.28	$C_{11}H_{200}$	NC
16	12.104	Hexadecanoic acid, methyl ester	2.87	270.5	$C_{17}H_{34}O_2$	Fatty Acids
17	12.15	Eudesma-4(15),7-dien-1.beta. -ol	0.69	220.35	$C_{15}H_{24}O$	Terpenoids
18	12.232	Platambin	1.22	238.37	$C_{15}H_{26}O_2$	Terpenoids
19	12.416	Pentadecanoic acid	3.77	242.4	$C_{15}H_{30}O_2$	Fatty Acids
20	12.562	Glyceryl diacetate 2-linolenate	1.47	436.6	$C_{25}H_{40}O_6$	NC
21	12.923	Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-	0.63	238.41	$C_{16}H_{30}O$	NC
22	13.396	9,12-Octadecadienoic acid, methyl ester	6.64	294.5	$C_{19}H_{34}O_2$	Fatty Acids
23	13.426	9-Octadecenoic acid (Z)-, methyl ester	2.51	296.5	$C_{19}H_{36}O_2$	Fatty Acids
24	13.458	Methyl linolenate	1.69	292.5	$C_{19}H_{32}O_2$	Fatty Acids
25	13.499	Phytol	1	296.5	$C_{20}H_{40}O$	Fatty Acids
26	13.525	Methyl 2-nonynoate	0.86	168.23	$C_{10}H_{16}O_2$	Fatty Acids
27	13.595	Methyl stearate	0.93	298.5	$C_{19}H_{38}O_2$	Fatty Acids
28	13.97	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	1.57	444.7	$C_{30}H_{52}O_2$	Fatty acids
29	16.624	2-Palmitoylglycerol	1.2	330.5	$C_{19}H_{38}O_4$	Glycerides
30	18.052	Glyceryl monolinoleate	3.02	354.5	$C_{21}H_{38}O_4$	Glycerides
31	22.287	beta.-Tocopherol	0.84	416.7	$C_{28}H_{48}O_2$	Vitamin
32	24.929	Benzenepropanoic acid, .alpha.-(1-hydroxyethyl)-.beta.-phenyl-	5.59	270.32	$C_{17}H_{18}O_3$	NC

NMW=No Molecular Weight, NMF=No Molecular Formula, NC=Not Classified

(1) **(2)** **(3)** **(4)**

(5) **(6)** **(7)** **(8)**



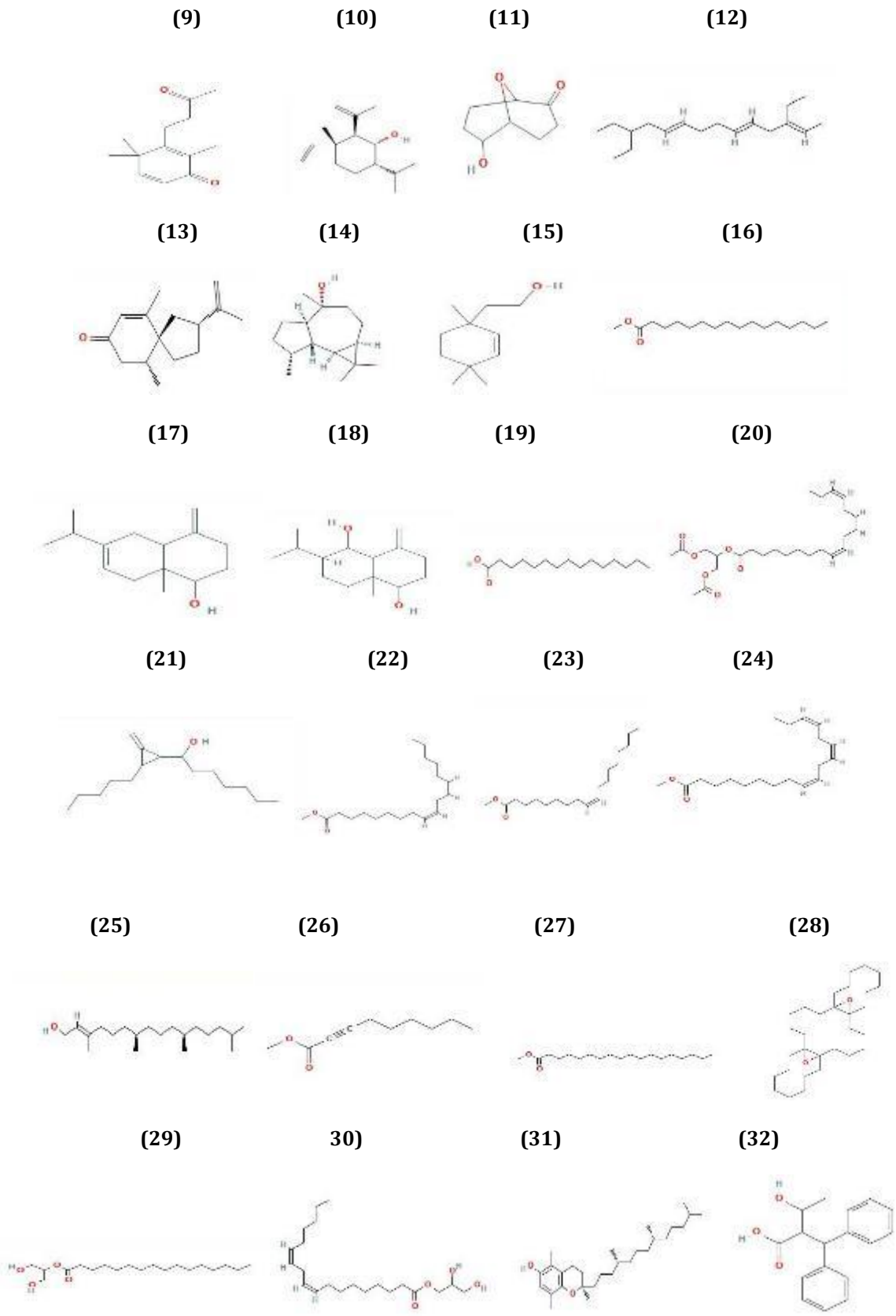


Figure 3 Structures of the GC-MS identified compounds (1-32) given in Table 4

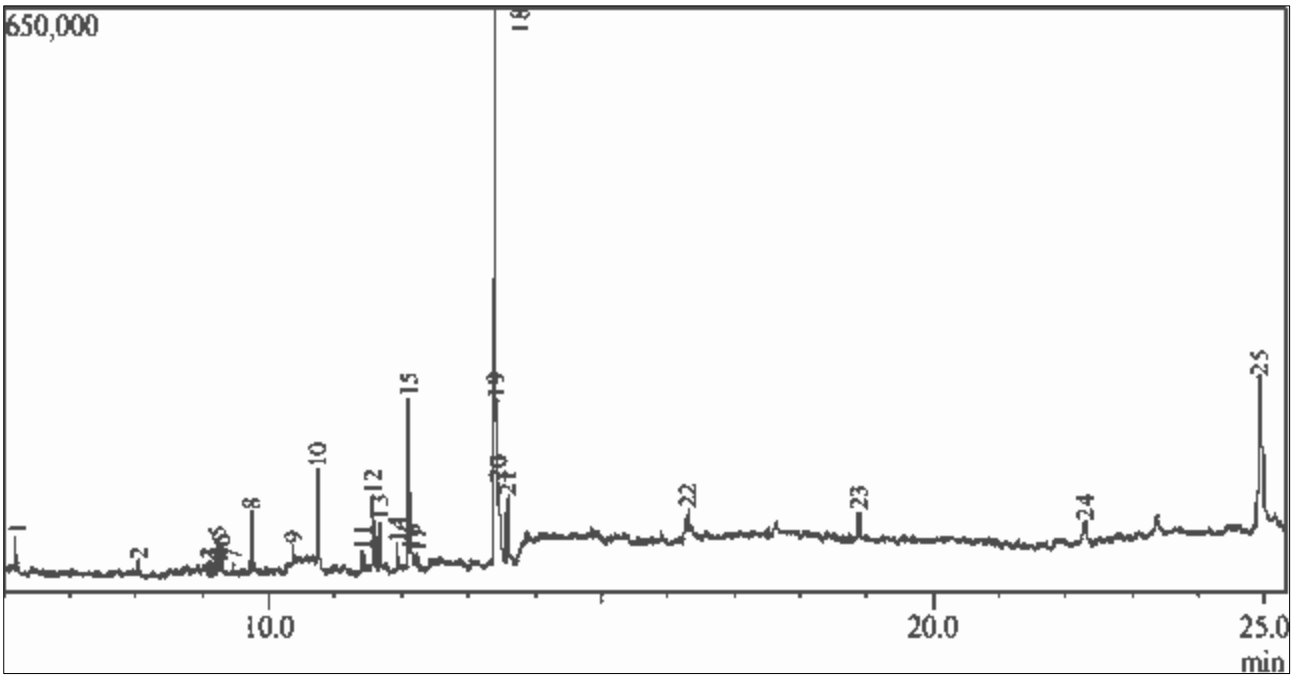


Figure 4 Chromatogram of the bioactive compounds in the n-hexane fraction of *S. aculeastrum* Dunal berries

Table 5 The bioactive compounds present in n-hexane fraction of *S. aculeastrum* Dunal berries identified by GC-MS

Peak No	Retention time (min)	Identity of compound	Peak Area (%)	Molecular Weight (g/mol)	Molecular Formula	Class
1	6.185	2,4-Dimethyl-2,4-pentanediol	1.61	132.2	$C_7H_{16}O_2$	NC
2	8.038	(-)-cis-.beta.-Elemene	0.53	204.35	$C_{15}H_{24}$	Terpenoids
3	9.11	beta-Humulene	0.76	204.35	$C_{15}H_{24}$	Terpenes
4	9.178	Cadina-1(10),4-diene	0.52	204.35	$C_{15}H_{24}$	Terpenes
5	9.231	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	1.49	306.5	$C_{20}H_{34}O_2$	Terpenes
6	9.281	6-epi-shyobunol	0.94	222.37	$C_{15}H_{26}O$	Terpenoids
7	9.459	2-(4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexyl)propan-2-ol	0.65	222.37	$C_{15}H_{26}O$	NC
8	9.741	2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	2.49	222.37	$C_{15}H_{26}O$	Terpenoids
9	10.374	Alpha.-Cadinol	0.91	222.37	$C_{15}H_{26}O$	Terpenoids
10	10.737	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]-	4.35	NMW	NMF	NC
11	11.41	Neophytadiene	1.11	278.5	$C_{20}H_{38}$	Alkene
12	11.563	Solavetivone	4.79	218.33	$C_{15}H_{22}O$	Terpenes

13	11.665	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]-	2.6	NMW	NMF	NC
14	11.929	2-(1,4,4-Trimethyl-cyclohex-2-enyl)-ethanol	1.3	168.28	$C_{11}H_{20}O$	NC
15	12.102	Hexadecanoic acid, methyl ester	7.85	270.5	$C_{17}H_{34}O_2$	Fatty acids
16	12.15	Alpha-Costol	1.58	220.35	$C_{15}H_{24}O$	Terpenoids
17	12.229	Platambin	1.18	238.37	$C_{15}H_{26}O_2$	Terpenoids
18	13.394	9,12-Octadecadienoic acid, methyl ester	25.21	294.5	$C_{19}H_{34}O_2$	Fatty acids
19	13.425	9-Octadecenoic acid, methyl ester	6.7	296.5	$C_{19}H_{36}O_2$	Fatty acids
20	13.456	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	5.05	278.4	$C_{18}H_{30}O_2$	Fatty acids
21	13.591	Methyl stearate	2.95	298.5	$C_{19}H_{38}O_2$	Fatty acids
22	16.311	1-Propanone, 1,3,3-triphenyl-	1.29	286.4	$C_{21}H_{18}O$	NC
23	18.886	Squalene	2.04	410.7	$C_{30}H_{50}$	Terpenes
24	22.295	.beta.-Tocopherol	1.85	416.7	$C_{28}H_{48}O_2$	Vitamins
25	24.922	Benzenemethanethiol, .alpha.-methyl-.alpha.-phenyl-	20.26	214.33	$C_{14}H_{14}S$	NC

NMW=No Molecular Weight, NMF=No Molecular Formula, NC=Not Classified

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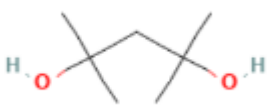
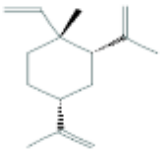
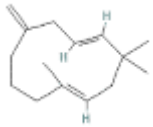
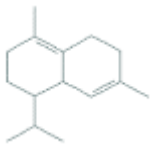

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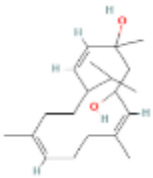
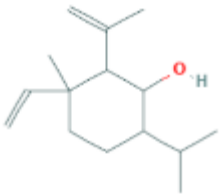
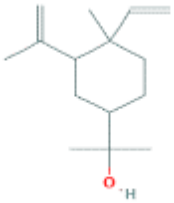
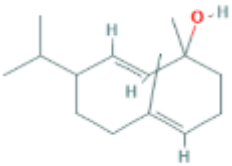

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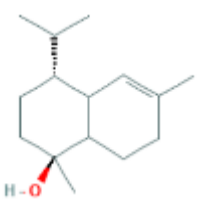
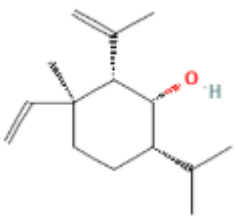
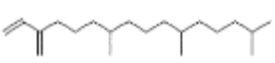
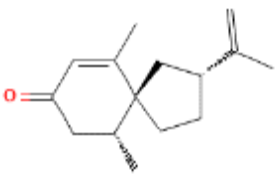

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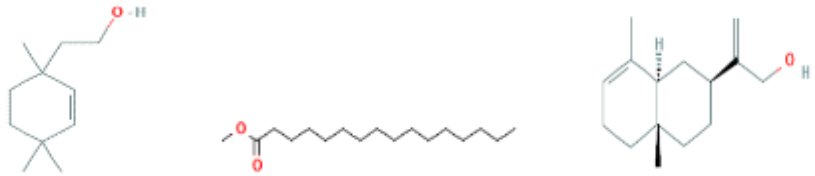

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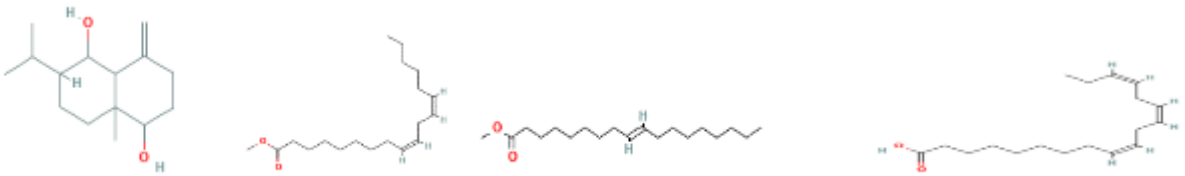

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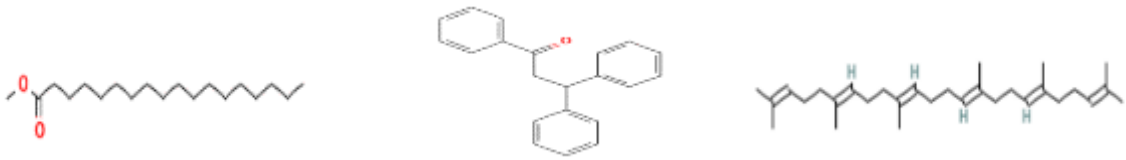

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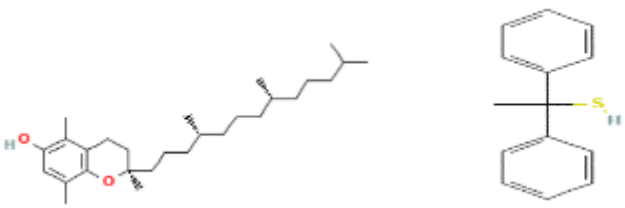


Figure 5 Structures of the GC-MS identified compounds (1-25) given in Table 5

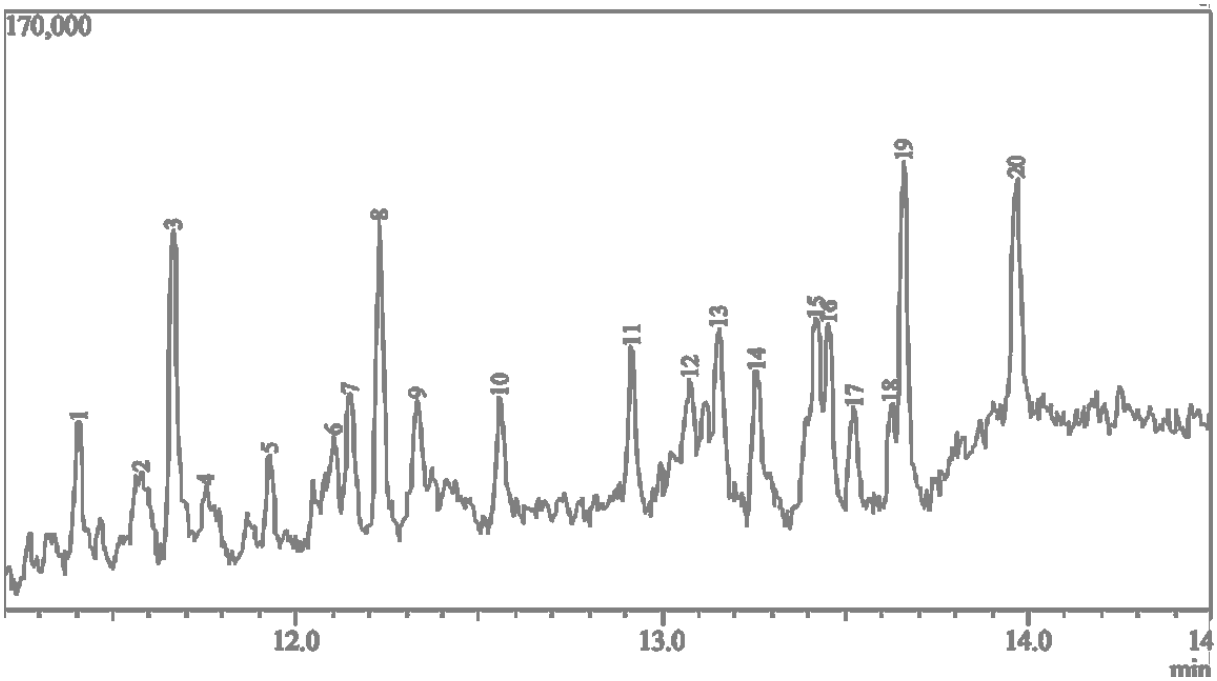


Figure 6 Chromatogram of the bioactive compounds in the DCM fraction of *S. aculeastrum* Dunal berries

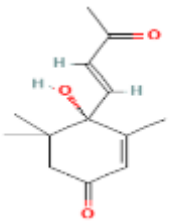
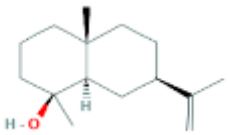
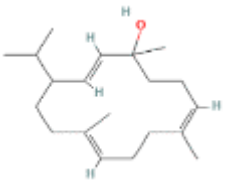
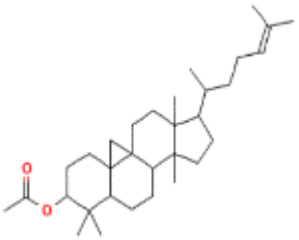
Table 6 The bioactive compounds present in DCM fraction of *S. aculeastrum* Dunal berries identified by GC-MS

Peak No	Retention time (min)	Identity of compound	Peak Area (%)	Molecular Weight(g/mol)	Molecular Formula	Class
1	11.407	Dehydrovomifoliol	4.3	222.28	$C_{13}H_{18}O_3$	Isoprenoids
2	11.578	Neointermedeol	4.32	222.37	$C_{15}H_{26}O$	Terpenoids
3	11.665	Thunbergol	10.46	290.5	$C_{20}H_{34}O$	Terpenoids
4	11.755	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	3.97	468.8	$C_{32}H_{52}O_2$	Terpenoids
5	11.928	Cyclopropa[d]naphthalen-3-one, octahydro-2,4a,8,8-tetramethyl-, oxime	0.8	235.36	$C_{15}H_{25}NO$	NC
6	12.105	DL-Norleucine	3.4	131.17	$C_6H_{13}NO_2$	amino acid
7	12.147	(1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	5.23	220.35	$C_{15}H_{24}O$	NC
8	12.229	Platambin	9.63	238.37	$C_{15}H_{26}O_2$	Terpenoids
9	12.331	(-)-Globulol	3.69	222.37	$C_{15}H_{26}O$	amino acid
10	12.557	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	3.2	238.37	$C_{15}H_{26}O_2$	NC
11	12.917	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	3.99	226.31	$C_{13}H_{22}O_3$	NC

12	13.077	(2R,3R,3aR,6R,8aS)-3,7,7-Trimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulen-2-ol	3.76	220.35	$C_{15}H_{24}O$	NC
13	13.155	Ingol 12-acetate	5.49	408.5	$C_{22}H_{32}O_7$	NC
14	13.257	Widdrol hydroxyether	4.01	238.37	$C_{15}H_{26}O_2$	NC
15	13.422	10-Methylundec-2-en-4-olide	6.26	196.29	$C_{12}H_{20}O_2$	NC
16	13.456	1-Heptadec-1-ynyl-cyclohexanol	5	334.6	$C_{23}H_{42}O$	NC
17	13.525	Widdrol hydroxyether	2.14	238.37	$C_{15}H_{26}O_2$	NC
18	13.625	Sinapic acid methyl ester	3.16	238.24	$C_{12}H_{14}O_5$	Carboxylic Acids
19	13.66	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo[3.3.3]undecane	9.93	249.4	$C_9H_{19}NO_3SSi$	NC
20	13.968	Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester	7.28	250.38	$C_{16}H_{26}O_2$	NC

NC=Not Classified

(1) (2) (3) (4)

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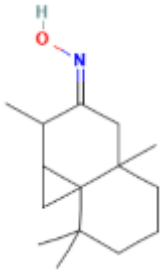
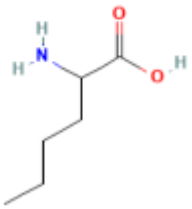
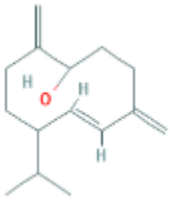
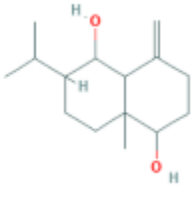

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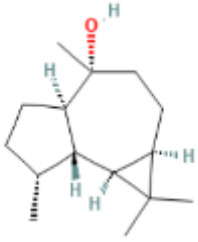
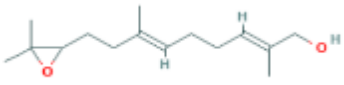
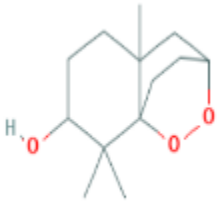
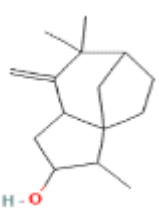

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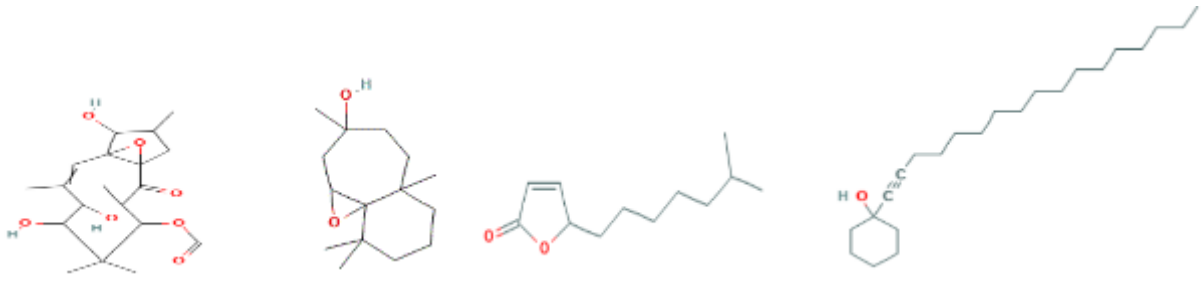

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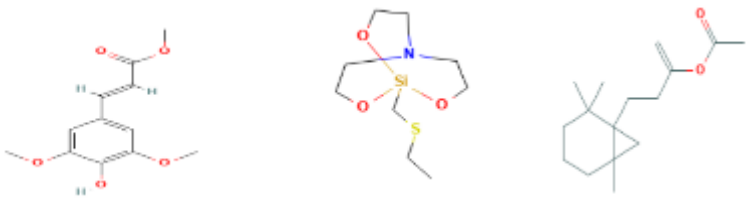


Figure 7 Structures of the GC-MS identified compounds (1-20) given in Table 6

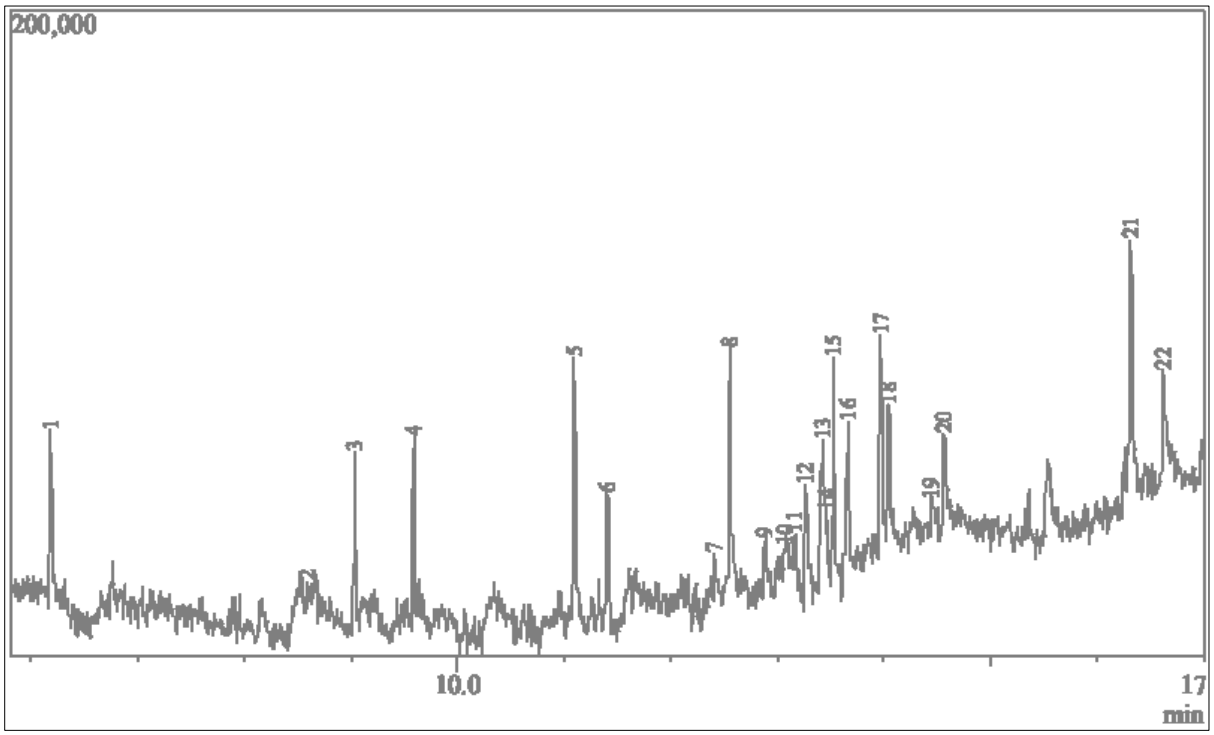


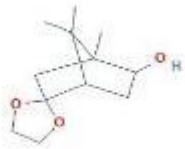
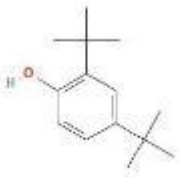
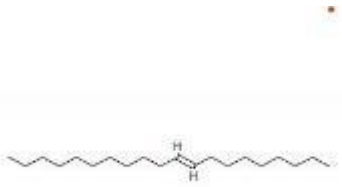
Figure 8 Chromatogram of the bioactive compounds in the Ethyl acetate fraction of *S. aculeastrum* Dunal berries

Table 7 The bioactive compounds present in ethyl acetate fraction of *S. aculeastrum* Dunal berries identified by GC-MS

Peak No	Retention time (min)	Identity of compound	Peak Area (%)	Molecular Weight (g/mol)	Molecular Formula	Class
1	6.185	2,4-Dimethyl-2,4-pentanediol	3.91	132.2	$C_7H_{16}O_2$	NC
2	8.59	2-Bornanol, 5,5-ethylenedioxy-	3.35	212.28	$C_{12}H_{20}O_3$	NC
3	9.035	2,4-Di-tert-butylphenol	4.56	206.32	$C_{14}H_{22}O$	Phenols
4	9.587	9-Eicosene, (E)-	4.43	280.5	$C_{20}H_{40}$	Fatty acids
5	11.096	Tetradecyl trifluoroacetate	6.64	310.39	$C_{16}H_{29}F_3O_2$	NC
6	11.406	Dehydrovomifoliol	4.3	222.28	$C_{13}H_{18}O_3$	Terpenoid
7	12.405	Undecanoic acid	3.35	186.29	$C_{11}H_{22}O_2$	Fatty Acids
8	12.555	1-Heneicosanol	6.44	312.6	$C_{21}H_{44}O$	Alcohol
9	12.88	5-Methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo [4.1.0	3.1	236.35	$C_{15}H_{24}O_2$	NC
10	13.07	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo [5.4.0.0(3,8)]undecane	3.71	220.35	$C_{15}H_{24}O$	NC
11	13.16	Tetracosa-2,6,14,18,22-pentaene-10,11-diol, 2,6,10,15,19,23-hexamethyl-	2	444.7	$C_{30}H_{52}O_2$	NC
12	13.262	Bicyclo [3.1.0] hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	3.67	170.25	$C_{10}H_{18}O_2$	NC
13	13.426	2-Dodecen-1-yl (-) succinic anhydride	6.57	266.38	$C_{16}H_{26}O_3$	NC
14	13.46	Bicyclo[2.2.1]heptane-2,3-diol, 1,7,7-trimethyl-, (exo,exo)-	2.63	266.38	$C_{16}H_{26}O_3$	NC
15	13.525	Widdrol hydroxyether	8.05	238.37	$C_{15}H_{26}O_2$	NC
16	13.662	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo [3.3.3] undecane	5.77	249.4	$C_9H_{19}NO_3SSi$	NC
17	13.97	Thunbergol	6.97	290.5	$C_{20}H_{34}O$	Terpenoids
18	14.045	9-Hexacosene	2.92	364.7	$C_{26}H_{52}$	Fatty acids
19	14.44	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	2.36	443.96	$C_{13}H_{39}O_5Si_6$	NC
20	14.561	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	4.33	204.35	$C_{15}H_{24}$	Terpenoids
21	16.314	1-Propanone, 1,3,3-triphenyl-	7.22	286.4	$C_{21}H_{18}O$	NC
22	16.621	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3.72	330.5	$C_{19}H_{38}O_4$	Glycerolipid

NC=Not Classified

(1) (2) (3) (4)

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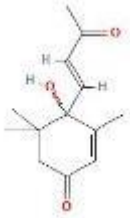


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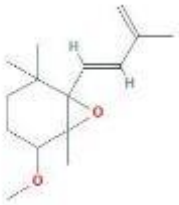

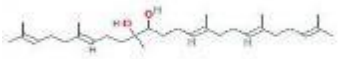
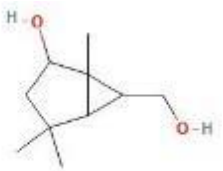

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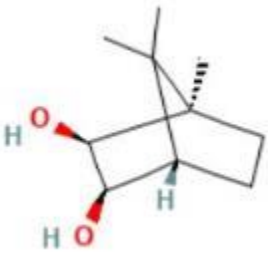
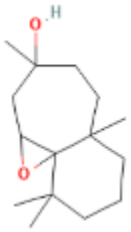


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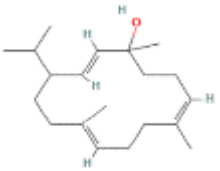

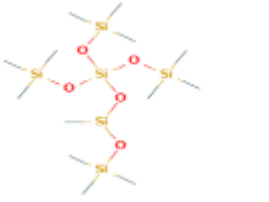

(20) (21) (22)

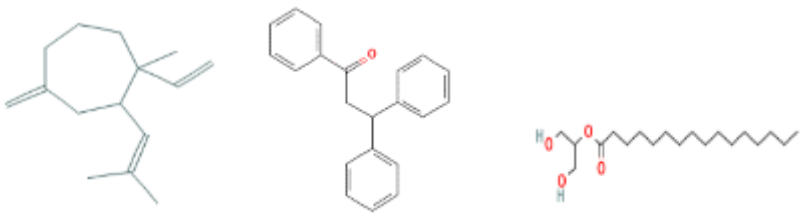

Figure 9 Structures of the GC-MS identified compounds (1-22) given in Table 7

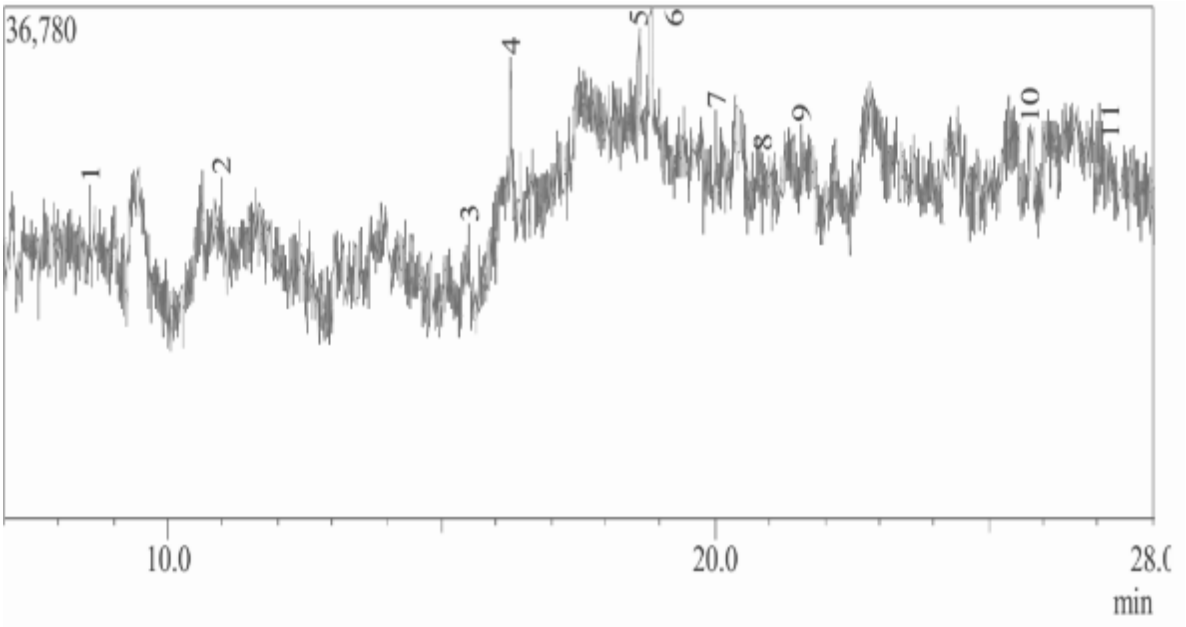

Figure 10 Chromatogram of the bioactive compounds in the n-butanol fraction of *S. aculeastrum* Dunal *berries*

Table 8 The bioactive compounds present in n-butanol fraction of *S. aculeastrum* Dunal berries identified by GC-MS

Peak No	Retention time (min)	Identity of compound	Peak Area (%)	Molecular Weight(g/ mol)	Molecular Formula	Class
1	8.575	Terephthalic acid, heptyl 3-methylbutyl ester	1.18	334.4	$C_{20}H_{30}O_4$	NC
2	10.965	N-(4-fluorophenyl)-3-(4-phenylpiperazin-1-yl)propanamide	1.03	327.4	$C_{19}H_{22}FN_3O$	Fluorinated aromatic substances
3	15.51	Octadecanoic acid, 16-oxo-, methyl ester	27.22	312.5	$C_{19}H_{36}O_3$	NC
4	16.26	Cyclononasiloxane, octadecamethyl-	5.38	667.4	$C_{18}H_{54}O_9Si_9$	NC

5	18.6	Ethyl homovanillate, TMS derivative	1.7	282.41	$C_{14}H_{22}O_4Si$	NC
6	18.828	17.alpha.Methyltestosterone	13.18	302.5	$C_{20}H_{30}O_2$	Hormonal antineoplastic agent
7	20.024	2,2,2-Trichloroethyl phosphorodichloridite	2.31	250.3	$C_2H_2Cl_5OP$	Organophosphorus Compounds
8	20.871	benzoic acid, 4-(2-benzoxazolyl)-	21.37	239.23	$C_{14}H_9NO_3$	NC
9	21.57	Ibuprofen	1.03	206.28	$C_{13}H_{18}O_2$	A non-steroidal anti-inflammatory agent with analgesic, antipyretic, and anti-inflammatory properties
10	25.757	Trinexapac-ethyl, TMS derivative	12.64	324.44	$C_{16}H_{24}O_5Si$	NC
11	27.223	Gamma.-Cyano-3-methyl-5,10 dihydrobenzo[f]indolizine	12.97	208.26	$C_{14}H_{12}N_2$	NC

NC=Not Classified

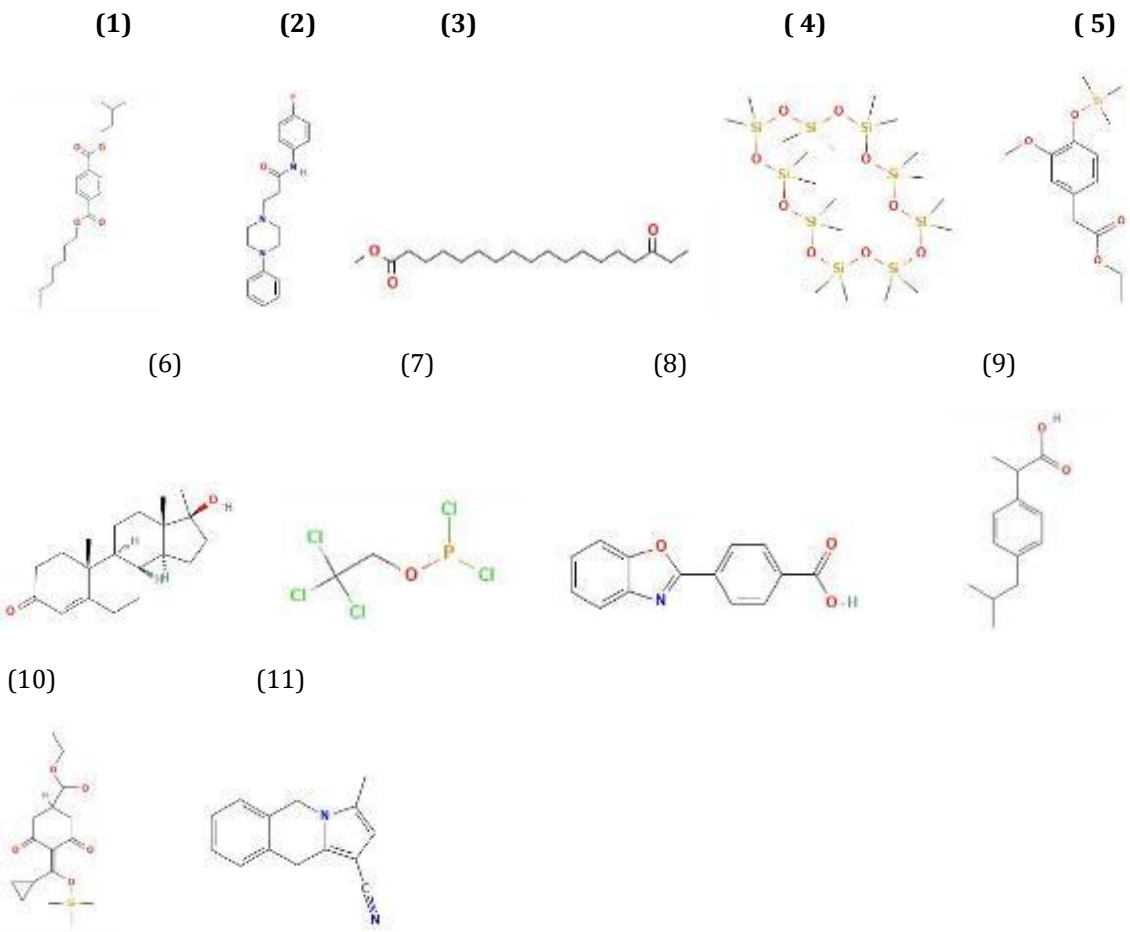

Figure 11 Structures of the GC-MS identified compounds (1-11) given in Table 8

4. Result and Discussion

The utilization of traditional medicine in primary health care has been in practice both in developed and developing countries [26]. About 80% of the population in developing countries use herbal medicines to meet their primary healthcare needs [27]. This probably could be due to the limited availability and high cost of conventional medicines [28]. The percentage yield of 430.69g of methanol whole extract of berries of *S.aculeastrum* Dunal was 9.57%w/w, while the yields of 19.4g of dried n-hexane fraction, 38.4g of dried DCM fraction, 6.4g of dried ethyl acetate fraction, 81.03g of dried n-butanol fraction and 21.2g of the freeze-dried aqueous fractions were 7.08%w/w, 14.03 %w/w, 2.33%w/w, 29.60%w/w and 7.74%w/w respectively (Table 2). With respect to the yield of the methanol whole extract, our result is in conformity with previous study where 1kg of ground berries of *S. aculeastrum* Dunal generated 94g of the extract implying a percentage yield of 9.4%, although they used 50% methanol (MeOH) and dichloromethane (CH_2CL_2) as solvents for extraction [29].

Our results of preliminary phytochemical screening revealed that the whole methanol extract of berries of *S. aculeastrum* Dunal contained abundance of all phytochemicals screened except quinones which were absent. The presence of glycosides in high amount, followed by moderate presence of alkaloids and steroids, and mild presence of terpenoids were confirmed in the n-hexane fractions, but flavonoids, phenols, tannins, quinones and saponins were absent. Regarding the DCM fraction, we confirmed the presence of alkaloids, glycosides, and tannins in high amounts, flavonoids and terpenoids were moderately present but there were no phenols, steroids, quinones and saponins. Screening of ethyl acetate fraction showed the moderate presence of alkaloids, glycosides, flavonoids and terpenoids while phenols, steroids, quinones and saponins were absent. For n-butanol fraction, alkaloids and terpenoids were highly present, glycosides, flavonoids, phenols, tannins, steroids were moderately present, but quinones and saponins were absent. The freeze-dried aqueous fraction revealed the presence of high amounts of alkaloids, flavonoids, phenols, terpenoids, with moderate amounts of steroids while glycosides, tannins and quinones were absent. There is an obvious absence of quinones in all extract and fractions implying that the berries of *S. aculeastrum* Dunal do not contain quinones (Table 3).

Our phytochemical screening results conform with the long existing information on the phytochemical constituents of the *Solanaceae* family, as secondary metabolites such as steroidal alkaloids have been isolated from more than 300 species of this plant family [30], [31]. Solamargine and solasonine are important alkaloids which have been found in Solanum species [32]. Plant alkaloids possess antioxidant, astringent and analgesic properties [33]. Glycosides such as β-solamarine and α-solamarine have also been identified in Solanum species [32] with important functions as glycosides are known to possess medicinal properties against different diseases including cancer [34].

Steroidal glycoalkaloids isolated from various species of *Solanum* have demonstrated pharmacological activities such as anti-trypanosomiasis [35]; antifungal [36], [37]; hepatoprotective [38], [39]; anti-seizure [40]; antiulcer [41]; cytoprotective [42]; neuropharmacological [43]; antimicrobial [44]; antioxidant [45] and anticancer activities [46]. More specifically, it was reported in a study that two steroidal alkaloids namely, tomatidine and solasodine isolated from berries of *Solanum aculeastrum* Dunal demonstrated significant anticancer effect against three cancer cell lines which include colonic adenocarcinoma (HT-29), cervical adenocarcinoma (Hela) and breast adenocarcinoma (MCF-7) cell lines [11].

Flavonoids are another important secondary metabolite with a range of medicinal properties including anticancer activities [47]; anti-inflammatory [48]; antioxidant activities [49] amongst others. Furthermore, the medicinal importance of tannins has also been well documented. Tannins are known to possess antibacterial, antioxidant, anti-inflammatory, antiviral, antiparasitic as swell as immunomodulatory effects [50]. The pharmacological relevance of the terpenoids have also been reported as they possess anti-inflammatory, analgesic, anticoagulant activities [51]; as well as antimicrobial, anti-hyperglycemic, antiviral, antiparasitic, and anticancer activities [52]. Saponins are well known for their antimicrobial, antioxidant and anti-inflammatory activities [53]; anticancer, cell apoptosis regulating activity, and play important roles in autophagy and angiogenesis [54]. Also, they have the ability to reduce cholesterol levels and interfere with multiplication process in cancer cells [55]. Phenols which are secondary metabolites synthesized by plants, play significant roles including protective effects in plants against pathogens, important in plant growth and signaling, as well as roles in management of cancers, diabetes, cardiovascular diseases, antiviral, and antiprotozoal activities [56], [57]

The GC-MS profiling of the methanol extract and n-hexane, DCM, ethyl acetate, and n-butanol fractions of the berries of *S. aculeastrum* Dunal revealed the presence of mixtures of straight chained aliphatic as well as ring-containing aromatic hydrocarbons. A total of 32 compounds were identified in the methanol whole extract of *S. aculeastrum* Dunal berries. These include an alkane (undecane), a fatty alcohol (2-Pentanol), an alcohol (Germacrene D-4-ol), a carbohydrate (D-

Arabinitol), 10 compounds classified as fatty acids [Eicosapentaenoic Acid; Hexadecanoic acid, methyl ester; Pentadecanoic acid; 9,12-Octadecadienoic acid, methyl ester; 9-Octadecenoic acid (Z)-, methyl ester; Methyl linolenate; Phytol; Methyl 2-nonynoate, Tricyclo [20.8.0.0(7,16)] triacontane,1(22),7(16)-diepoxy-], 5 compounds belonging to the terpenes/terpenoids [Shyobunol; Solavetivone; Globulol; Eudesma-4(15),7-dien-1.beta. -ol; Platambin and Methyl stearate], 2 compounds classified as glycerides (2-Palmitoylglycerol and Glyceryl monolinoleate), 1 compound classified as vitamin (beta.-Tocopherol) and 11 miscellaneous unclassified compounds (Table 4, Figure 3).

The GC-MS profiling of the n-hexane fraction of *S. aculeastrum* Dunal berries revealed the presence of 25 bioactive compounds, which include 11 bioactive compounds classified as terpenes/terpenoids [(-)-cis-.beta.-Elemene; beta-Humulene; Cadina-1(10),4-diene;4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-; 6-epi-shyobunol; 2E,4S,7E)-4-Isopropyl-1,7 dimethylcyclodeca-2,7-dienol; Solavetivone; Alpha-Costol; Platambin and Squalene], 1 alkene (Neophytadiene), 5 fatty acids (Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid, methyl ester; 9-Octadecenoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- and Methyl stearate], I compound classified as vitamin (beta.-Tocopherol) and 7 unclassified compounds (Table 5, Figure 5). Profiling of the DCM fraction of the extract identified 20 bioactive compounds including 1 compound classified as isoprenoid (Dehydrovomifoliol), 4 terpenoids [Neointermedeol; Thunbergol; 9,19-Cyclolanost-24-en-3-ol, acetate, (3. beta.)-; and Platambin], 2 amino acids [DL-Norleucine and (-)-Globulol], 1 carboxylic acid (Sinapic acid methyl ester) and 12 unclassified compounds (Table 6, Figure 7).

A total of 22 bioactive compounds were identified in the ethyl acetate fraction. These include 1 compound belonging to the phenols (2,4-Di-tert-butylphenol), 3 compounds belonging to the fatty acids [9-Eicosene, (E)-; Undecanoic acid and 9-Hexacosene], 1 compound classified as alcohol (1-Heneicosanol), 3 terpenoids [Dehydrovomifoliol; Thunbergol and Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-], 1 compound classified as glycerolipid [Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester] and 13 unclassified compounds (Table 7, Figure 9). The n-butanol fraction contained 11 identified compounds which 1 compound belonging to the fluorinated aromatic substances [N-(4-fluorophenyl)-3-(4-phenylpiperazin-1-yl)propenamide],1 compound classified as hormonal antineoplastic agent (17.alpha.Methyltestosterone), 1 compound belonging to the organophosphorus compounds (2,2,2-Trichloroethyl phosphorodichloridite), 1 compound belonging to non-steroidal anti-inflammatory agent with analgesic, antipyretic, and anti-inflammatory properties (Ibuprofen) and 7 unclassified compounds (Table 8, Figure 11).

A study has conducted GC-MS profiling of the bioactive constituents of the berries of *S.aculeastrum* Dunal which involved only the n-hexane fraction where the authors reported 16 identified bioactive compounds [58]. Our study involved a comprehensive and exhaustive profiling of the bioactive compounds present in the berries of *S. aculeastrum* Dunal through GC-MS analysis of the methanol whole extract, n-hexane, ethyl acetate, DCM and n-butanol fractions which demonstrated the presence of a plethora of bioactive compounds compared to what have been identified previously. This has therefore further broadened the knowledge on the bioactive components of berries of the plant that may be responsible for their diverse pharmacological activities.

Interestingly, the previous study on the GC-MS profiling of the n-hexane fraction yielded 12 compounds belonging to alkanes/alkenes, 3 compounds belonging to the fatty acids and 1 compound categorized as miscellaneous. However, our GC-MS profiling of the n-hexane fraction of berries of *S. aculeastrum* Dunal, yielded 25 bioactive compounds including 11 bioactive compounds belonging to the terpenes/terpenoids, 1 alkene, 5 fatty acids, I compound classified as vitamin and 7 unclassified compounds (Table 5, Figure 5). Another important observation is the presence of 4 isomers (compounds with peak numbers 6,7, 8 and 9) of the identified bioactive compounds in the n-hexane fraction which have same molecular formula ($C_{15}H_{26}O$) but different structures (Table 5, Figure 5). Also, we realized that the compound with peak number 7 [(2-(4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexyl) propan-2-ol] was not classified from the PubChem source to the best of our knowledge. However, having found that it is an isomer of compounds with peak numbers 6, 8 and 9 which are classified as terpenoids, we could therefore infer that the compound with peak number 7 [2-(4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexyl) propan-2-ol] could be a terpenoid as well. The composition of plant compounds and molecules would also be influenced by its habitat and geographical location which may explain the difference in our findings compared to the previous study.

These observations on the n-hexane fraction coupled with the diverse bioactive compounds identified in the methanol whole extract and other fractions represent a monumental improvement on the previous study. Also, apart from the fact that some compounds are yet to be classified, the molecular weights and formula of two compounds with peak number 10 [(Cyclohexanol,3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R (1. alpha.,2. alpha.,3. beta.,6. alpha.)]-)] and peak number13 [(Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-,[1R-(1. alpha.,2. alpha.,3. beta.,6. alpha.)]-)] were not identified in the n-hexane fractions. A close observation revealed a striking similarity in these two compounds both in names and structures but with different peak areas and retention

times. We also observed that few similar compounds were present across some of the extracts. For instance, the compound, Dehydrovomifoliol (terpenoid) was found in both the DCM and ethyl acetate fractions. Similarly, the compound, Platambin (terpenoid) was identified in the methanol extract, DCM and n-hexane fractions. Generally, majority of the GC-MS identified bioactive compounds across all solvent's extracts are terpenoids, followed by fatty acids. This may explain most of the activities demonstrated by *S. aculeastrum* Dunal berries, bearing in mind, the potentials of these secondary metabolites especially the terpenoids [51].

Furthermore, we noticed the presence of ibuprofen identified in the n-butanol fraction. Ibuprofen belongs to the non-steroidal anti-inflammatory agents with analgesic, antipyretic, and anti-inflammatory properties, which may therefore explain its reported anti-nociceptive and anti-inflammatory activities [59]. The identification of 17-alpha-Methyltestosterone, classified as hormonal antineoplastic agent in the n-butanol fraction may be partly responsible for the reported anticancer activities of the *S. aculeastrum* Dunal berries in previous studies [11], [14]. The profiling of the bioactive compounds in medicinal plants provides more information and better understanding of their demonstrated pharmacological properties. This study, to the best of our knowledge could represent one of the most exhaustive screening and profiling of the berries of *S. aculeastrum* Dunal.

5. Conclusion

Giving the increasing patronage of medicinal plants in management of various ailments across the globe, the need for screening and identification of their bioactive compounds remains paramount. Our study has further elaborated on the bioactive compounds present in berries of *S. aculeastrum* Dunal, to aid robust understanding and study of its pharmacological activities.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no competing interests.

Statement of ethical approval

Approval was obtained from School of Graduate Studies (SGS), Maseno University. Ethical clearance was obtained from Maseno University Ethical Review Committee (MUERC) with approval number: MUERC/00898/20. Research permit was also obtained from the National Commission for Science, Technology and Innovation (NACOSTI), Kenya, with license number: NACOSTI/P/21/8440.

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Authorship Contribution Statement

All authors collaborated in the conduct of this research. GCP, BG, PGM, and JO conceptualized the study. GCP wrote the protocol and collected the data. GCP, MJ and IJLL organized and prepared the results. GCP wrote the first draft of the manuscript. The final manuscript was read and approved by all authors.

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