

Possible Cytotoxic Activity Analysis of Diethyl Ether Extract of *Vaccinium varingiaefolium* (Blume) Miq. Leaves by GC-MS Method

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Abstract

Background: *Vaccinium varingiaefolium* (Blume) Miq. of Ericaceae is an endemic plant that grows well near volcano craters spreading from east to west of Indonesia. Its young leaves have been used as food and traditional medicines, however there has been very little information on bioactive compounds. **Objective:** To identify bioactive compounds with cytotoxic activity of diethyl ether extract of *Vaccinium varingiaefolium* leaves by GC-MS method.

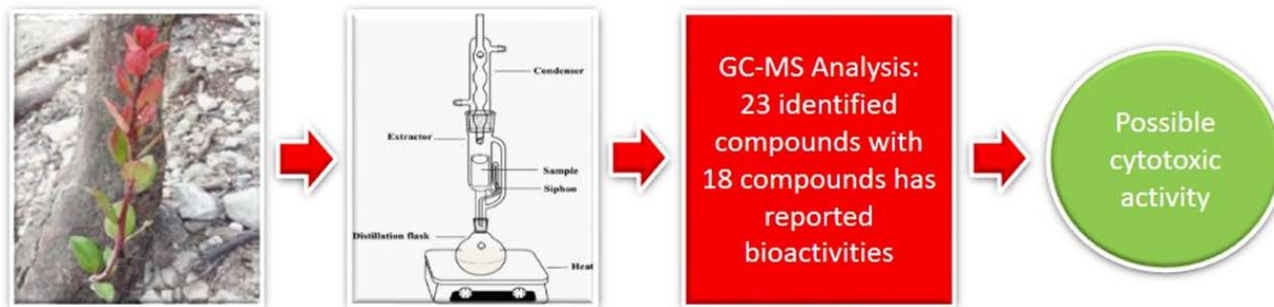
Material and Methods: Fresh leaves were collected from Mount Tangkuban Parahu, North Bandung, Indonesia, identified, dried, powdered, then extracted with diethyl ether using Soxhlet apparatus. Extracts were dried and identified by GC-MS method.

Results: The GC-MS analysis identified 23 compounds such as organosilicon, sesquiterpene alcohol, heterocyclic, fatty acid ester, fatty acid, ketone, lipid, plasticizer, alkane, triterpenoid, and pentacyclic triterpenoid based on the mass spectral library with various activities such as analgesic, antiallergic, antibacterial, anticancer, anticoronary, antidiabetic, antifungal, antihyperlipidemic, anti-inflammatory, antimicrobial, antioxidant, antipyretic, antispasmodic, and antiviral based on previous studies.

Conclusion: Based on the results, *Vaccinium varingiaefolium* leaves contains bioactive compounds with possible cytotoxic activity.

Key words: Bioactive compounds, Diethyl ether extract, GC-MS, Possible cytotoxic activity, *Vaccinium varingiaefolium* (Blume) Miq.

Pictorial Abstract



INTRODUCTION

Vaccinium is a genus of Ericaceae family having about 450 species worldwide. Its berries and leaves in certain places are being consumed as food and herbal medicines. *V. myrtillus* and *V. macrocarpon* from Europe and North America, respectively, for examples are used as functional foods.^[1]

Indonesia has one species of *Vaccinium* genus, namely *Vaccinium varingiaefolium* (Blume) Miq. or Cantigi as the common local name. The plants grow well especially near the crater of volcanoes spreading from Sumatra, Java, Kalimantan, Sulawesi, Nusa Tenggara Timur, and to Papua Island.^[2-7] They are found abundantly in the area at high altitude about 1500-3300 m above sea level.^[8]

Traditionally, local people use *V. varingiaefolium* leaves and berry as food and herbal medicines. Report on phytochemical profile of *V. varingiaefolium* is still very little. One study by Forney *et al.* identified 34 floral volatile compounds using GC-MS method as pollinator attracting.^[9]

Another study by Kosasih *et al.* identified 15 volatile compounds in ethyl acetate extract of *V. varingiaefolium* leaves using GC-MS method. Moreover, the extract showed strong cytotoxic activity on Leukemia 1210 cells.^[10]

The purpose of this study was to identify bioactive compounds of diethyl ether extract of *V. varingiaefolium* leaves whether contained probable cytotoxic activity by GC-MS method.

MATERIALS AND METHODS

Plant collection, identification, and extraction

V. varingiaefolium leaves were collected from Mount Tangkuban Parahu, North Bandung, West Java province, Indonesia. Plants were botanically identified and authenticated at Pusat Penelitian Biologi, Lembaga Ilmu Pengetahuan Indonesia, Cibinong Science Center, Cibinong, West Java, Indonesia.

Two grams of young leaf samples (three replicates each) were dried at 60 °C, powdered by grinding in a mortar, and extracted with 100 mL of diethyl ether for 8 hours in a Soxhlet apparatus. Extracts were evaporated at 40 °C under reduced pressure.^[11]

GC-MS Analysis.

The analysis by GC-MS was carried out at the Regional Health Laboratory, Jakarta, Indonesia with slightly modification^[12]. The diethyl ether extract of *V. varingiaefolium* leaves was carried out using GC-MS system of 7890A/5975 with auto sampler and Mass Selective Detector and Chemstation data system (Agilent Technologies, USA). Setting of the system was electron energy of 70 eV and ionization mode of electron impact with capillary column of HP Ultra 2 (length of 30 m, internal diameter of 0.20 mm, and film thicknesses of 0.11 µm). Oven initial temperature was at 80 °C (no hold), increased at 3 °C/min to 150 °C (1 min), and finally increased at 20 °C/min to 280 °C. Injection port temperature was 250 °C, ion source temperature of 230 °C, interface temperature of 280 °C and quadrupole temperature of 140 °C. Carrier gas used was helium with column mode of constant flow, column flow of 1.2 mL/min, injection volume of 5 µL, and split of 8:1.

Identification of compounds.

Mass spectrum GC-MS was interpreted using the database library of National Institute Standard and Techniques (NIST) of W8N08.L.

RESULTS AND DISCUSSION

Results of the GC-MS analysis of diethyl ether extract of *V. varingiaefolium* leaves identified 23 bioactive compounds as seen on the chromatogram (Figure 1). Table 1 presented the bioactive compounds based on the mass spectral library, their retention time (RT), concentration (%), molecular formula, molecular weight, peak area (%), and compound nature. The predominant identified compounds were pentacyclic triterpenoid, fatty acid ester, and alkanes.

Among the identified compounds, 18 compounds revealed 90-99% similarity indices with NIST08 Library, namely cyclotrisiloxane, hexamethyl- (91%), alpha bisabolol (91%), hexadecanoic acid, methyl ester (99%), hexadecanoic acid (99%), 9-octadecenoic acid (Z), methyl ester (99%), 11-octadecenoic acid, methyl ester (99%), octadecanoic acid, methyl ester (99%), .beta.-monoolein (90%), 1,2-benzenedicar-boxylic acid, mono(2-ethylhexyl) ester (91%), eicosane (98%), nonacosane (97%), heptacosanol (95%), hentriacontane (98%), .gamma.-sitosterol (99%), beta-amyrin (94%), alpha-amyrin (97%), friedelanol (91%), and friedelin (95%). These similarities were high enough as an indicator of mass spectral similarity measures. Figure 2 presented 2D chemical structures of bioactive compounds obtained from the PubChem, an open chemistry database at the National Institutes of Health (NIH), USA.

Other important results were shown in Table 2 presenting identified compounds and their bioactivities of the diethyl ether extract of *V. varingiaefolium* leaves. Based on previous studies, those bioactive compounds provided various bioactivities. Moreover, the predominant compounds with the percentage of > 5% showed cytotoxic activity, anti-inflammatory, and antioxidant. The results implied that the extract exhibit medicinal values, especially as a candidate of anticancer.

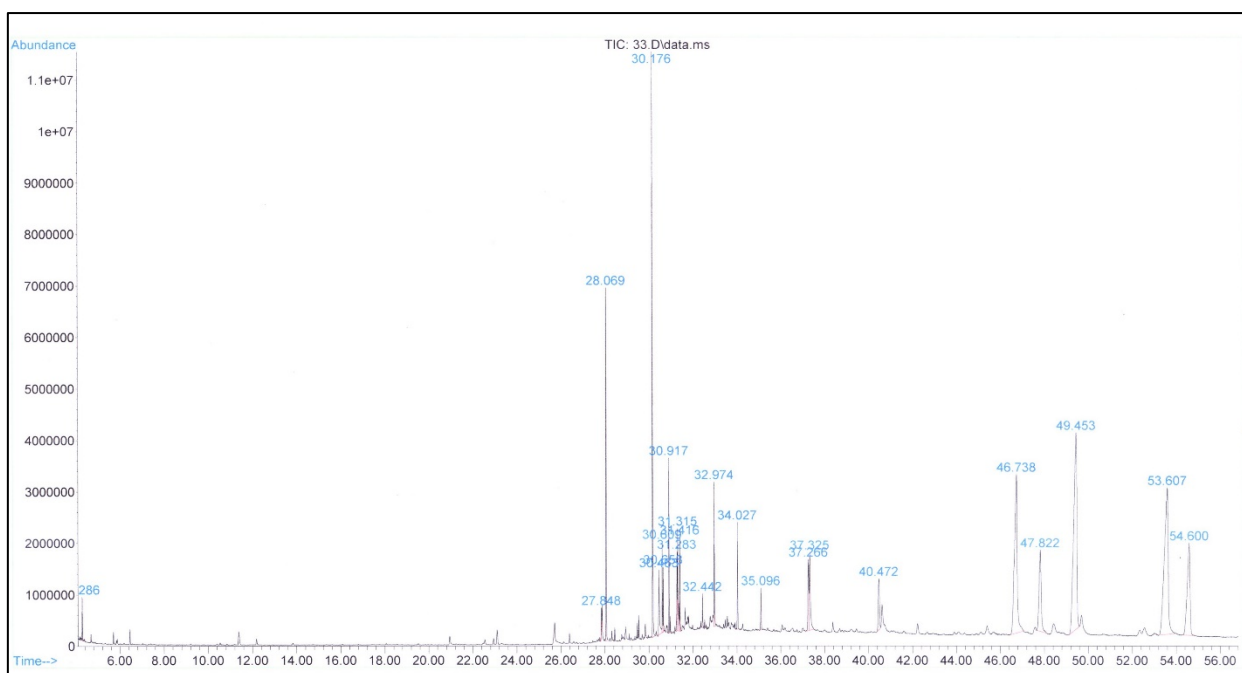


Figure 1: GC-MS chromatogram of diethyl ether extract of *V. varingiaefolium* leaves.

Table 1: Phytochemicals identified in the diethyl ether extract of *V. varingiaefolium* leaves by GC-MS method.

No.	Rt (minute)	Compound name	Molecular formula	Molecular weight	Peak area (%)	Compound nature
1	4.287	Cyclotrisiloxane, hexamethyl-	C ₆ H ₁₈ O ₃ Si ₃	222.46	0.49	Organosilicon
2	27.848	Alpha bisabolol	C ₁₅ H ₂₆ O	222.37	0.19	Sesquiterpene alcohol
3	28.069	2H-tetrazole, 5-(thiopen-2-yl) methyl-	C ₆ H ₆ ON ₄ S	166.21	5.18	Heterocyclic
4	30.179	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	7.11	Fatty acid ester
5	30.461	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	1.70	Fatty acid
6	30.606	3-decen-5-one, 2-methyl-	C ₁₁ H ₂₀ O	168.28	1.06	Ketone
7	30.654	Sulfurous acid, cyclohexylmethyl heptadecyl ester	C ₂₄ H ₄₈ O ₃ S	416.70	0.72	Ester
8	30.916	3-decen-5-one, 2-methyl-	C ₁₁ H ₂₀ O	168.28	1.91	Ketone
9	31.282	9-octadecenoic acid (Z), methyl ester	C ₁₉ H ₃₆ O ₂	296.50	1.08	Fatty acid ester
10	31.316	11-octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.50	1.62	Fatty acid ester
11	31.413	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298.50	1.04	Fatty acid ester
12	32.440	7-octen-2-one	C ₈ H ₁₄ O	126.20	0.47	Ketone
13	32.971	.Beta.-mono-olein	C ₂₁ H ₄₀ O ₄	356.50	2.25	Lipid
14	34.026	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C ₁₆ H ₂₂ O ₄	278.34	1.59	Plasticizer
15	35.095	Eicosane	C ₂₀ H ₄₂	282.50	0.78	Alkane
16	37.267	Nonacosane	C ₂₉ H ₆₀	408.8	2.02	Alkane
17	37.322	Heptacosanol	C ₂₇ H ₅₆ O	396.7	2.71	Alcohol
18	40.473	Hentriacontane	C ₃₁ H ₆₄	436.8	1.98	Alkane
19	46.741	.Gamma.-sitosterol	C ₂₉ H ₅₀ O	414.7	13.95	Triterpenoid
20	47.824	Beta-amyrin	C ₃₀ H ₅₀ O	426.7	5.76	Pentacyclic triterpenoid
21	49.451	Alpha-amyrin	C ₃₀ H ₅₀ O	426.7	19.69	Pentacyclic triterpenoid
22	53.609	Friedelanol	C ₃₀ H ₅₂ O	428.7	18.34	Pentacyclic triterpenoid
23	54.602	Friedelin	C ₃₀ H ₅₀ O	426.7	8.36	Pentacyclic triterpenoid

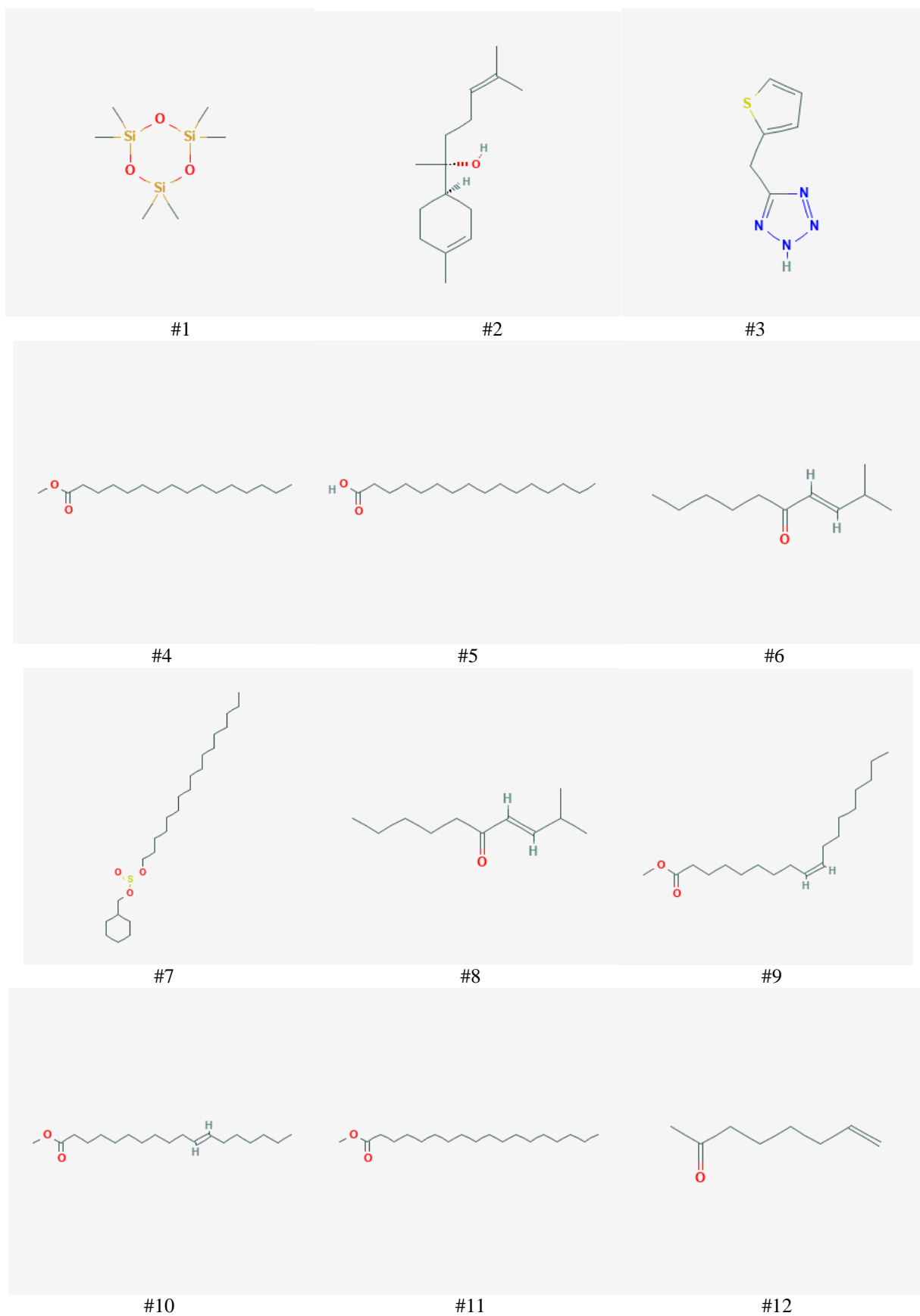


Figure 2: Chemical structures of bioactive compounds of diethyl ether extract of *V. varingaefolium* leaves.

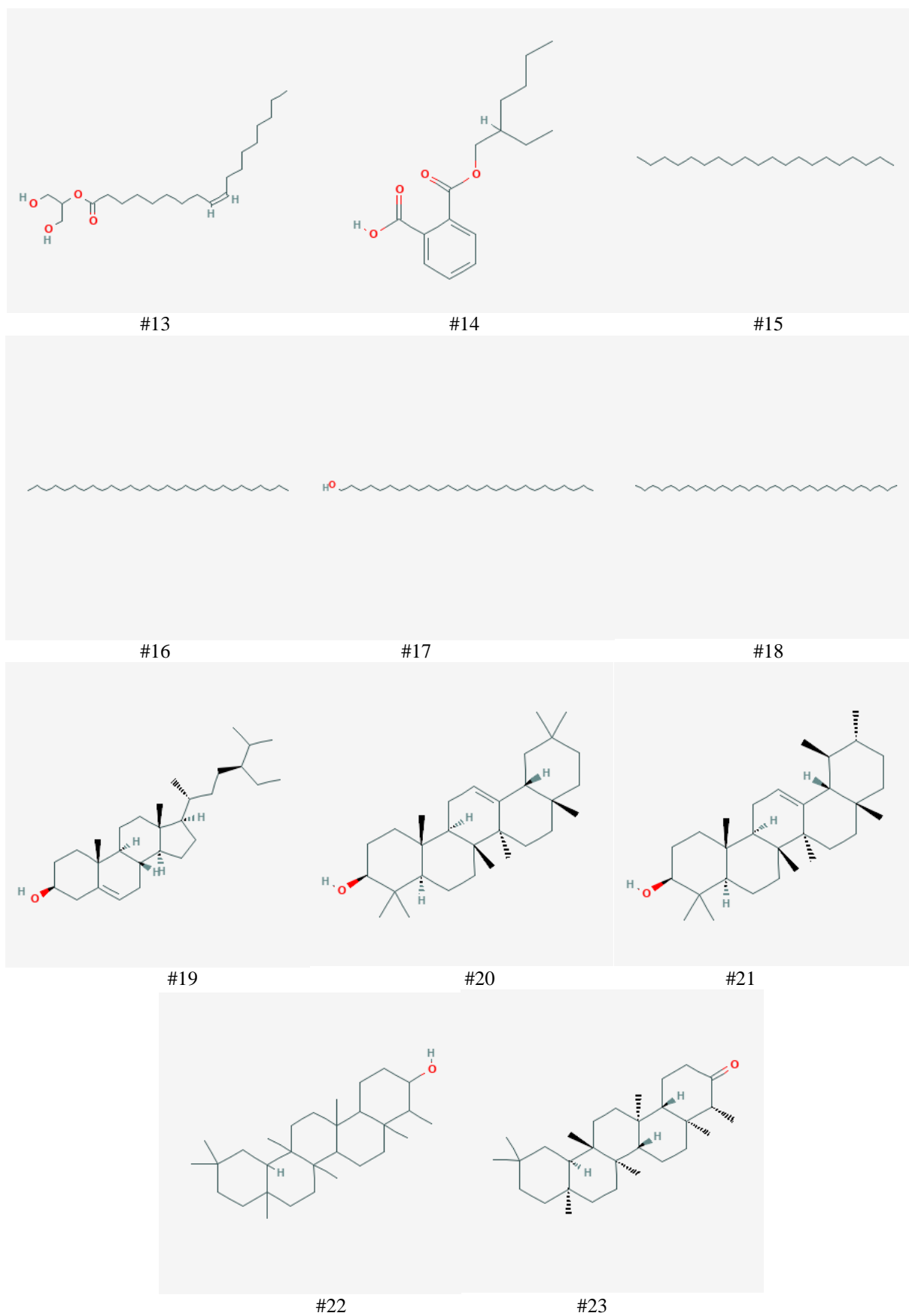


Figure 2 : Chemical structures of bioactive compounds of diethyl ether extract of *V. varingaefolium* leaves.

Table 2: Identified compounds and biological activities of the diethyl ether extracts of *V. varingiaefolium* leaves.

No	Compound name	Biological activity	References
1	Cyclotrisiloxane, hexamethyl-	Antiplasmodial, antimicrobial, antioxidant	13, 14, 15
2	Alpha bisabolol	Leukemia, penetration enhancer, anti-inflammatory, antispasmodic, antiallergic	16, 17, 18
3	2H-tetrazole, 5-(thiopen-2-yl) methyl-	Antibacterial, antioxidant	19
4	Hexadecanoic acid, methyl ester	Antioxidant, antifungal	20
5	Hexadecanoic acid	Cytotoxic, antibreast cancer, antioxidant	21, 22, 23
6	3-decen-5-one, 2-methyl-	No report	-
7	Sulfurous acid, cyclohexylmethyl heptadecyl ester	No report	-
8	3-decen-5-one, 2-methyl-	No report	-
9	9-octadecenoic acid (Z), methyl ester	Antifungal, antioxidant, antimicrobial, anticancer	20, 24, 25
10	11-octadecenoic acid, methyl ester	Antidiarrheal, anti-inflammatory, anti-hypercholesterolemia, cancer preventive, hepatoprotective, antistaminic, antiacne, alpha reductase inhibitor, antieczemic, antiandrogenic, anticoronary, antiarthritic	26, 27
11	Octadecanoic acid, methyl ester	Antiviral, Antifungal, antibacterial, cytotoxic, antimicrobial.	20, 28, 29
12	7-octen-2-one	No report	-
13	.Beta.-mono-olein	Antioxidant, anti-atherosclerotic, protein glycation inhibitor, blood lipid-lowering	30, 31, 32
14	1,2-benzenedicar-boxylic acid, mono(2-ethylhexyl) ester	Cytotoxic, antimicrobial, antioxidant, antiinflammatory. Antidiabetic	33, 34, 35
15	Eicosane	Antifungal, antimicrobial	36, 37
16	Nonacosane	Antimicrobial	38, 39
17	Heptacosanol	No report	-
18	Hentriacontane	Antiinflammatory, antitumor, antimicrobial	40, 41
19	.Gamma.-sitosterol	Antidiabetic, antibreast cancer, antihyperlipidemic	42, 43, 44
20	Beta-amyrin	Antimicrobial, antifungal, antiinflammatory, antiulcer, xanthine oxidase inhibitor, antiproliferative, antiplatelet, antiplamodium, antinociceptive, antidepressant.	45, 46, 47
21	Alpha-amyrin	Antimicrobial, antifungal, antiinflammatory, antiviral, anticancer, antinociceptive, antiulcer	45, 46, 47, 48
22	Friedelanol	Antiviral, anti-inflammatory, anticancer	49, 50, 51
23	Friedelin	Analgesic, anti-inflammatory, antipyretic, antidiarrheal, antioxidant, antidiabetic, antimycobacterial, antihyperlipidemic, anticancer	52, 53, 54, 55, 56, 57

CONCLUSION

This study is the first report of volatile bioactive compounds profile of diethyl ether extract of *V. varingiaefolium* leaves by GC/MS methods. The findings show various compounds with various bioactivity based on previous studies. This study implies that the extract is possible as a candidate of anticancer. For further study, it is strongly recommended to isolate, purify, and characterize bioactive compounds with various cancer cells.

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Conflict Of Interest -The authors declare that there is no conflict of interest.

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