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Characterization of Aqueous Extract of *Moringa Oleifera* leaves using GC-MS Analysis.

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~~ABSTARCT~~ **ABSTRACT**

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Background

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The Evaluation of plants for its bioactive compounds is of great importance to researchers because of their therapeutic properties

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which can be harnessed to serve as alternatives to antibiotics or the development of novel drugs to fight human infectious diseases.

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Moringa oleifera is a member of the Moringaceae family with nutritional and medicinal properties.

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Aim:

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To identify the bioactive constituents present in *Moringa oleifera* leaves using Gas chromatography-~~M~~mass spectrometry.

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Methodology:

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The bioactive compounds were extracted using the Soxhlet method of extraction and ~~waswere~~ analyzed using ~~Gas chromatography-~~

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~~mass-~~spectrometry.

Comment [H2]: Gas chromatography-mass spectrometry

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Results

16 The chromatogram of the GC-MS analysis used to identify the bioactive constituents present in the aqueous extract of *Moringa*
17 *oleifera* leaves extract showed the presence of seventy-eight compounds. This includes 1H-Indene, 2-pyridinyl)-methyl ester (9.82%),
18 Anthracene, 9-(2-propenyl) (6.72%), 2,6-Lutidine 3,5-dichloro-4-dideiylthio (5.67%) and various other compounds which were
19 identified asat low level.

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20 Conclusions

21 The GC-MS analysis of *Moringa oleifera* showed the presence of bioactive compounds. These compounds can be used in the
22 production of new antimicrobials due to their valuable medicinal and therapeutic properties.

24 **Keywords:** GC-MS analysis, chemical constituents, *Moringa oleifera*, aqueous extract, bioactive compounds

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1. INTRODUCTION

Moringa oleifera is a Moringaceae species native to Africa, Arabia, South Asia, South America, the Himalaya region, India, Pakistan, the Pacific, and Caribbean Islands.⁽¹⁾ *Moringa* species are well-studied plant herbs because of their nutritional and medicinal properties. The most widely cultivated species in the Moringaceae monogenic family are *Moringa oleifera* and *Moringa stenopetala*.⁽¹⁾ *Moringa oleifera* has spread naturally in many tropical and subtropical regions of the world. The plant is referred to by a number of names such as horseradish tree, drumstick tree, Ben oil tree, miracle tree, and "Mother's best friend".⁽²⁾ They are acknowledged in folk medicine for their efficacy in the treatment of many different illnesses. They have anti-helminthic, antibiotic, detoxifying, and immune-building properties and have been used to treat malaria.⁽³⁾ *Moringa*, which is high in nutritional value and high in vegetable oil, is used as a food and medicinal plant in Asia. This is due to the presence of proteins, vitamins, and various phenolic compounds.⁽⁴⁾ Regardless, all portions of the *Moringa* tree are edible and have been eaten by humans for many years.

Moringa plant (*Moringa oleifera*) has been the focus of extensive research because of its numerous applications and well-known bactericidal potentials.⁽⁵⁾ Previous studies have revealed some bioactive ingredients from different parts of the plant. Faizi *et al.* (1994) [6]⁽⁶⁾ reported that *Moringa oleifera* leaf ethanolic extract contains niazirin, niazirin, and niazininins A and B. Manguro and Lemmen, (2007) [7]⁽⁷⁾ also reported that benzoic acid, gallic acid, and beta benzaldehyde were isolated from a methanolic extract of *Moringa oleifera* leaves. Igwe *et al.* [8] (2015)⁽⁸⁾ reported the presence of phytochemicals present in the methanolic leaves extract of *Moriga oleifera* using Gas chromatography mass-spectrometry (GC-MS). Nitesh *et al.* [9](2021)⁽⁹⁾ showed the phytochemical content of *Moringa oleifera* leaves extract by GC-MS and its free radical scavenging ability. Other studies by Jabeen *et*

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51 | *al.* [10](2008)⁽⁴⁹⁾ revealed that seeds and leaves extracts (and extracts) show activity against different species of fungi such as
52 | *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Epidermophyton floccosum* and so on. Some of them are
53 | purely anthropophilic dermatophytes. These extracts have bactericidal and/or bacteriostatic activity against *Staphylococcus aureus*,
54 | *Vibrio cholerae*, *Klebsiella pneumoniae* V. parahaemolyticus, *Enterococcus faecalis*, *Salmonella enteritidis*, *Aeromonas caviae*,
55 | *Pasteurella multocida*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa* [10].⁽⁴⁹⁾ In order to utilize and make the best use of the
56 | available natural wealth, phytochemical screening is critical in identifying new sources of therapeutically and industrially valuable
57 | compounds with medicinal significance. As a result, the purpose of this study is to determine the phytochemical constituents of
58 | *Moringa oleifera* leaf extracts using GC–MS.

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59 | MATERIALS AND METHODS

60 | 2.1 Collection of plant materials

61 | Fresh *Moringa oleifera* leaves were taken from a mature healthy tree in a farm house at Owerri, Imo state, Nigeria. The leaves were
62 | identified and authenticated by Professor Cyril Duru of Crop Science and Technology Department, Federal University of
63 | Technology, Owerri, Imo state, Nigeria. The plant was deposited in the herbarium of Department of Crop Science and Technology,
64 | Federal University of Technology, Owerri, with the voucher number CSTM/FUTO/4451.

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67 | **2.2 Extraction of plant materials**

68 | *Moinga oleifera* leaves were first~~ly~~ washed with sterilized water to remove dirt and debris. Jensen's Soxhlet method was used to
69 | prepare the plant extract ⁽¹¹⁾. The samples were dried in 500ml clean boiling flasks for 30 mins in an oven set to 105--110 degrees
70 | Celsius. After that, it was placed inside a desiccator and allowed to cool. 100g of the sample was weighed and poured into the soxhlet
71 | thimble. To aid in extract filtering, the extraction thimble was lightly plugged with cotton wool, and the boiling flask was filled with
72 | 300ml of ethanol. The soxhlet device was put together and allowed to reflux at 600 degrees Celsius for 4 hours. After carefully
73 | removing the thimble, a volumetric flask was filled with the extracts and allowed to cool. The volumetric flasks contents were
74 | transferred to a rotatory evaporator in order to separate the solvent from the oil.

75 | **2.3 Extraction of phytochemicals**

76 | The extract (1g) was weighed and placed in a test tube, then 25ml of ethanol was added. On a hotplate set to 600 degrees Celsius, the
77 | test tube was given 90 minutes to react. The reaction product in the test tube was transferred to a separator funnel after the reaction
78 | period. The tube was thoroughly cleaned with 20ml of ethanol, 10ml of cold water, 10ml of hot water, and 3ml of hexane before being
79 | moved into the funnel. The leaves extract was mixed~~with~~ and washed three times with a 10ml aqueous solution of 10% v/v ethanol.
80 | After being dried with anhydrous sodium sulfate, the solvent was evaporated. The sample was dissolved in 1000ul of pyridine, with
81 | 200ul transferred into the vial for analysis.

82 | **2.4 Gas Chromatography--Mass spectrometry (GC-MS)**

83 The GC–MS analysis of bioactive constituents from *Moringa Oleifera* leaf extracts was carried out using Agilent Technologies GC
84 systems with the GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with the HP-5MS column (30
85 m in length 250 m in diameter 0.25 m in film thickness). GC–MS spectroscopic detection required a method for ionizing electrons
86 with high energy electrons (70 eV). The carrier gas was pure helium gas (99.995% purity) with a flow of 1 mL/min. The initial
87 temperature was set at 50–150-°C, with a 3-°C/min increase rate and a holding time of about 10 minutes. Finally, the temperature was
88 raised to 300-°C at 10-°C each minute. In a splitless mode, one microliter of the prepared 1% extracts diluted with respective solvents
89 was injected. Based on the peak area produced in the chromatogram, the relative quantity of chemical compounds present in the
90 extracts was expressed as a percentage.

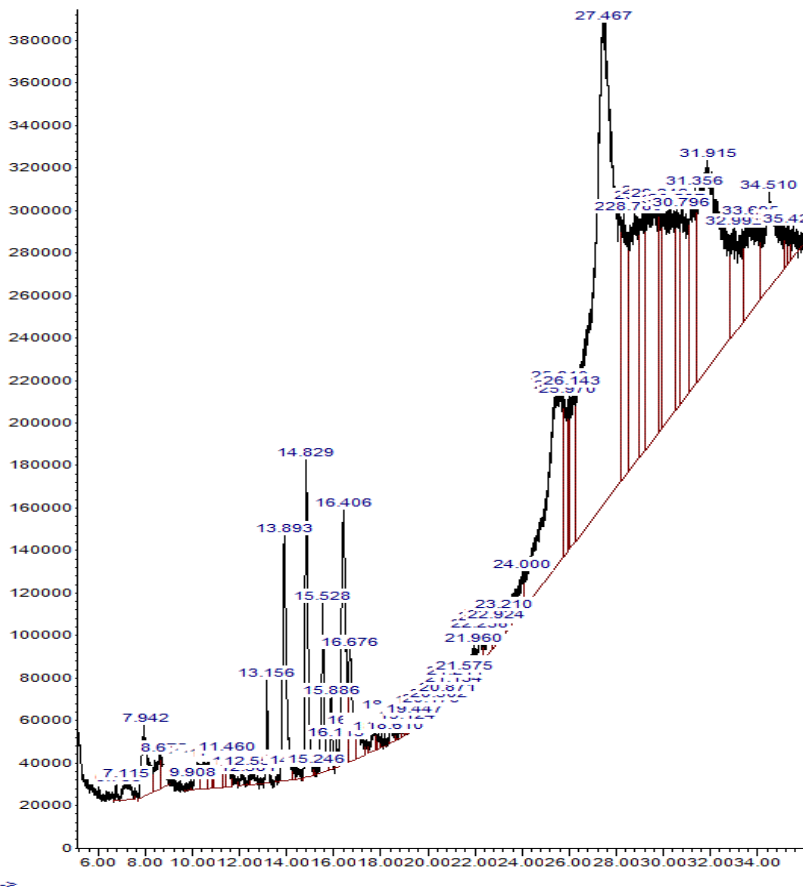
91 **2.5 Identification of bioactive constituents**

92 Bioactive compounds extracted from *Moringa oleifera* extracts were identified using the Gas Chromatography retention time on [the](#)
93 HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems)
94 ⁽¹²⁾

95

96 **3.0 RESULTS**

97 The GC-MS analysis of the aqueous fraction of *Moringa oleifera* leaves extract revealed 78 peaks, which depicts the presence of 78
98 bioactive compounds. The chromatogram is shown in Figure 1 while the bioactive compounds with their retention time (RT), peak
99 areas (%), molecular formula and molecular weight (MW) are shown in Table 1. The mass spectra of a few of the compounds with
100 high concentrations are shown in Figure 2-7. Among the seventy-eight compounds identified, The main abundant compounds present
101 in *Moringa oleifera* were 1H-Indene, 2-butyl-5-hexyloctahydro (25.02%), 4H-Pyran-3-carboxylic acid, 2-anono-5-cyano-6-ethyl-4-
102 (3-pyridinyl)-methylester (9.82%), Anthracene, 9-(2-propenyl) (6.72%), 2,6-Lutidine 3,5-dichloro -4-dideilythio (5.67%), 1-
103 Hydroxy-3-methoxypropan-2-yl oleate (5.19%), Thymol, TBDMS derivative (5.02%), 2-Ethylacridine (3.28%),
104 Benzo(h)quionoline, 2,4-dimethyl (2.95%), 6-chloro-1-ethyl-4-oxo-N(pyridon-4-yl methyl) quinolone-3-carboxamide (2.86%), 1,2
105 -Benzenediol, 3,5-bis(1,1-dimethylethyl) (2.63), Propanamide, N(3-methoxyphenyl)-2,2-dinethyl (2.59%), Cis-13-Octadecenoic
106 acid (2.46%), 3-pyridinamine, 2-(4-methyl-4H-1, 2,4-triazol-3-yl) thio (2.28%), Cyclopenteno(4,3-b) tetrahydrofuran, 3-[(4-
107 methyl-5-oxo-3 phenylthio) (2.11%) and various other compounds were identified as low level.



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Time-->

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FIGURE 1: shows GC-MS chromatogram of aqueous extract of *Moringa oleifera*

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111 **Table 1: Bioactive Compounds Identified in *Moringa oleifera* leaves by GC-MS**

S.NO	Name of Compound	Retention time (RT)	Peak area	Molecular formula	Molecular weight(MW)
1	2-Fluorobenzyl alcohol	6.555	0.05	C ₇ H ₇ F ₀	126
2	(1S, 2R, 4R, 7R) -4-Iso propyl-7-methyl-3,8dioxatricyclo (5.1.0.0 ² , 4) octane	7.115	0.29	C ₁₀ H ₁₆ O ₂	168
3	1-Nitro -2-acetamido 1, 2-dodeoxy-d-mannitol	7.942	0.95	C ₈ H ₁₆ N ₂ O ₇	252
4	Butanoic acid, 3hydroxyl-, ethylester	8.586	0.34	C ₆ H ₁₂ O ₃	132
5	2H- Azepin-2-acetamido--1, 2dodeoxy-d-mannitol	7.942	0.95	C ₆ H ₁₂ O ₃	132
6	6Acetyl-beta-d-mannose	9.908	0.05	C ₈ H ₁₄ O ₇	222
7	Undec -10ynoic acid, undecyl ester	10.206	0.28	C ₂₂ H ₁₄₀ O ₂	336
8	Cyclononene	10.466	0.29	C ₉ H ₁₆	124
9	Dodecyl proylether	10.705	0.18	C ₁₅ H ₃₂ O	228
10	Decanoic acid, 2,3dihydroxypropylester	10.819	0.12	C ₁₃ H ₂₆ O ₄	246
11	Undecylenic acid	11.068	0.31	C ₁₁ H ₂₀ O ₂	184
12	Trehalose	11.377	0.16	C ₅ H ₂₂ O ₁₁	3
13	d-lyxose	11.460	0.22	C ₅ H ₁₀ O ₅	150
14	Undec-10ynoic acid, tetradecylester	12.076	0.09	C ₂₅ H ₄₆ O ₂	378
15	Cydopentadecanone, 2hydroxy	12.381	0.04	C ₁₅ H ₂₈ O ₂	240
16	Myristoleic acid	12.550	0.10	C ₁₄ H ₂₆ O ₂	226
17	Hexadecanoic acid, methylester	13.156	0.38	C ₁₇ H ₃₄ O	270

18	n-Hexadecanoic acid	13.893	1.97	C ₁₇ H ₃₄ O ₂	270
19	Z,Z-4,16 Octadecadien-1-ol acetate	14.292	0.05	C ₂₀ H ₃₆ O ₂	308
20	3-Dodecen-1-ol	14.477	0.06	C ₁₂ H ₂₄ O	184
21	Heptadeca-1,9-dien-4, 6-diyn-3-ol	14.829	1.93	C ₁₇ H ₂₄ O ₁₁	244
22	9octadecenal	15.184	0.01	C ₁₈ H ₃₄ O	266
23	Cyclopropaneoctanal, 2-octyl	15.246	0.01	C ₁₉ H ₃₆ O	280
24	11Octadecenoic acid, methyl ester	15.528	1.05	C ₁₉ H ₃₆ O ₂	296
25	Methyl stearate	15.886	0.31	C ₁₉ H ₃₈ O ₂	298
26	Trichloroacetic acid, undec-2-enylester	16.113	0.12	C ₁₃ H ₂₁ Cl ₃ O ₂	315
27	Cis-13-Octadecenoic acid	16.406	2.46	C ₁₈ H ₃₄ O ₂	282
28	Octadecanoic acid	16.676	0.95	C ₁₈ H ₃₆ O ₂	284
29	Cycloeicosane	16.955	0.37	C ₂₀ H ₄₀	280
30	11-Dodecen -1-ol trifluoroacetate	17.714	0.16	C ₁₄ H ₂₃ F ₃ O ₂	280
31	(z)-9octadecen 1-olide	17.768	0.03	C ₁₈ H ₃₂ O ₂	280
32	1,3,12Nonadecatriene	17.910	0.06	C ₁₉ H ₃₄	262
33	Oleic acid	18.012	0.08	C ₁₈ H ₃₄ O ₂	282
34	E-11-Hexadecenal	18.431	0.16	C ₁₆ H ₃₀ O	238
35	9,17-Octadecadienal, (z)	18.610	0.01	C ₁₈ H ₃₂ O	264
36	9-octadecenoic acid, methylester (E)	18.856	0.08	C ₁₉ H ₃₆ O ₂	296
37	Oleic acid18.902	18.902	0.04	C ₁₈ H ₃₄ O ₂	282
38	(Z)-Tetradec-11-en-1-yl 2,2,2-trifluoroaceta	18.997	0.02	C ₃ H ₃ F ₃ O ₂	128

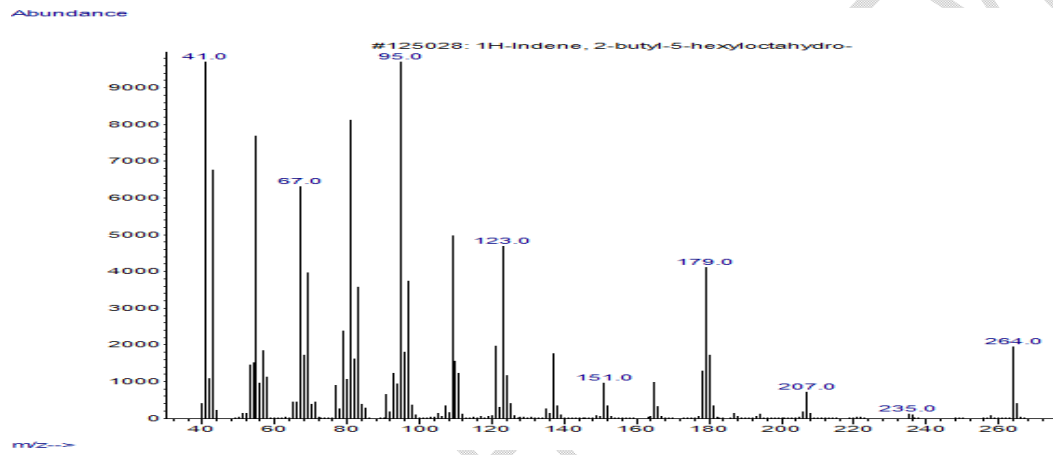
39	9-Octadecenoic acid (z), -2-hydroxyl-1-(hydroxymethyl) ethylester	19363	0.06	C ₂₁ H ₃₈ O ₄	354
40	9-Octadecanoic acid (z), 2-3-dihydroxypropyl ester	19.363	0.06	C ₂₁ H ₄₀ O	356
41	2-methyl, z-z-3, 13-octadecadienol	19.447	0.05	C ₁₉ H ₃₆ O	280
42	9-octadecenoic acid (E)	19.840	0.11	C ₁₈ H ₃₄ O ₂	282
43	Z-10-Methyl-11-tetradecen -1-01 proponate	20.178	0.06	C ₁₈ H ₃₄ O ₂	282
44	Octadec -9-enoic acid	20.502	0.09	C ₁₈ H ₃₄ O ₂	282
45	6-octadecenoic acid, (z)	20.780	0.07	C ₁₈ H ₃₄ O ₂	282
46	9- octadecenoic acid, (E)-Ethylolate	20.871	0.01	C ₂₀ H ₃₈ O ₂	310
47	1-Cyclohexylnonene	21.134	0.07	C ₂₁ H ₂₈	208
48	Carbon acid, propargyl 2,2,2-trichloroethyl	21.211	0.03	C ₆ H ₅ Cl ₃ O ₃	231
49	6- Octadecenoata	21.517	0.06	C ₁₈ H ₃₄ O ₂	282
50	n- propyl 9-octadecenoate	21.575	0.01	C ₂₁ H ₄₀ O ₂	
51	Octasiloxane , 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca methyl	21.960	0.01	C ₁₆ H ₄₈ O ₇ S ₁₈	577
52	Octadec -9-enoic acid	22.238	0.33	C ₁₈ H ₃₄ O ₂	282
53	Propyleneglycolmonoleate	22.536	0.36	C ₂₁ H ₄₀ O ₃	340
54	9- octadecenoic acid(z), 2-hydroxy-1-(hydroxyl methyl) ethjester	22.821	0.13	C ₂₁ H ₄₀ O ₄	354
55	Oleic acid, 3- hydroxyl propyl ester	22.924	0.07	C ₂₁ H ₄₀ O ₃	340
56	Chloromethyl 6-chloroundecanoate	23.210	0.21	C ₁₂ H ₂₂ C ₁₂ O ₂	269
57	Acetamode, 2-chloro N -(3-cyano - 4,6- dihydro-	24.000	0.82	C ₁₉ H ₁₃ CIN ₂ O ₂	336

	4,4,6,6-tetramethylthieno (2,3-c) furan – 2yl)				
58	Anthracene, 9-(2-propenyl)	25.610	6.72	C ₁₇ H ₁₄	218
59	4,7,7-trimethybicyclo (2.2-1) heptan-2- one o-allyloxime	25.777	1.13	C ₁₂ H ₂₁ NO	207
60	Pyridone-3-carboxanide, oxime, x1-2-trifluoromethyl phenyl	25.970	0.77	C ₁₃ H ₁₀ F ₃ O ₂ N ₃ O	281
61	2,6-lutidone, 3,5-dichloro -4-cyclohexylthio	26.143	1.35	C ₁₉ H ₃₁ CL ₂ O ₂ N ₅	376
62	1H-Indene, 2-butyl-5-hexyloctahydro	27.467	25.02	C ₁₉ H ₃₆	264
63	2-Ethylacridine	28.294	3.28	C ₁₅ H ₁₃ N	207
64	Thymol, TBDMS derivative	28.780	5.02	C ₁₃ H ₂₂ N ₅ Si	222
65	3-pyridinamine, 2- (4-methyl -4H-1, 2-4- triazol-3-yl) thio]	29.170	2.28	C ₈ H ₉ N ₅ S	207
66	2,6-Lutidine 3,5-dichloro -4-dodecylthio	29.470	5.67	C ₁₉ H ₃₁ CL ₂ NS	376
67	2-(n-propyl) oxgbenzylideneacetophenoneacetophenone	29.843	1.72	C ₁₁ H ₁₄ O	162
68	1- Hydroxy-3-methoxypropan -2-yloleate	30.337	5.19	C ₂₂ H ₄₂ O ₄	370
69	Demecolcine	30.597	1.41	C ₂₁ H ₂₅ NO ₅	371
70	Benzo(h)quionoline, 2,4-dimethyl	30.796	2.95	C ₁₅ H ₁₃ N	207
71	6- chloro-1- ethyl-4-oxo-N(pyridon -4-yl methyl) quinolone-3-carboxamide	31.356	2.86	C ₁₇ H ₁₅ CIN ₄ O ₂	342
72	4H-Pyran-3- carboxylic acid, 2-anono-5-cyano-6-ethyl-4-(3- pyridinyl)-methylester	31.915	9.82	C ₁₆ H ₁₆	284

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114 The mass spectra of some active components identified in the aqueous extract of *Moringa oleifera* were identified based on the GC-
115 retention time and compared with computer software data of standards.

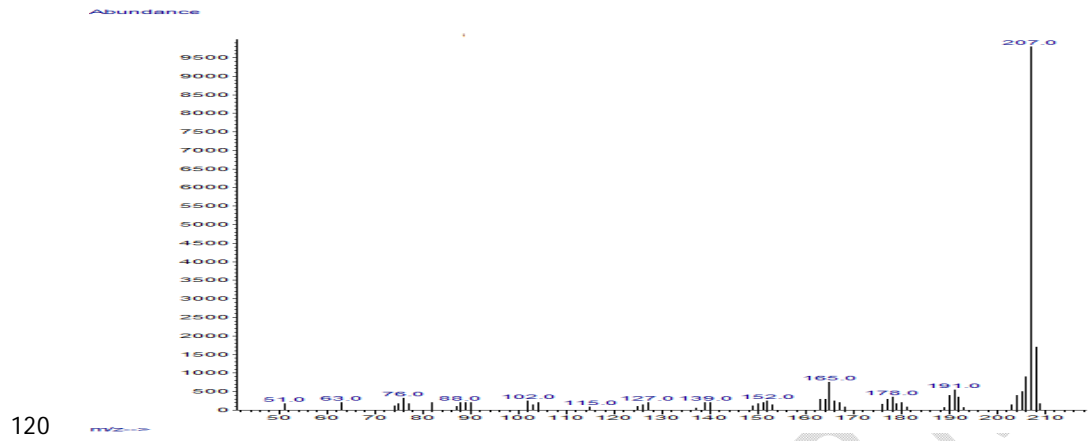
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118 **Figure 2: shows the mass spectra of 1H-Indene, 2-butyl-5-hexalocahydro**

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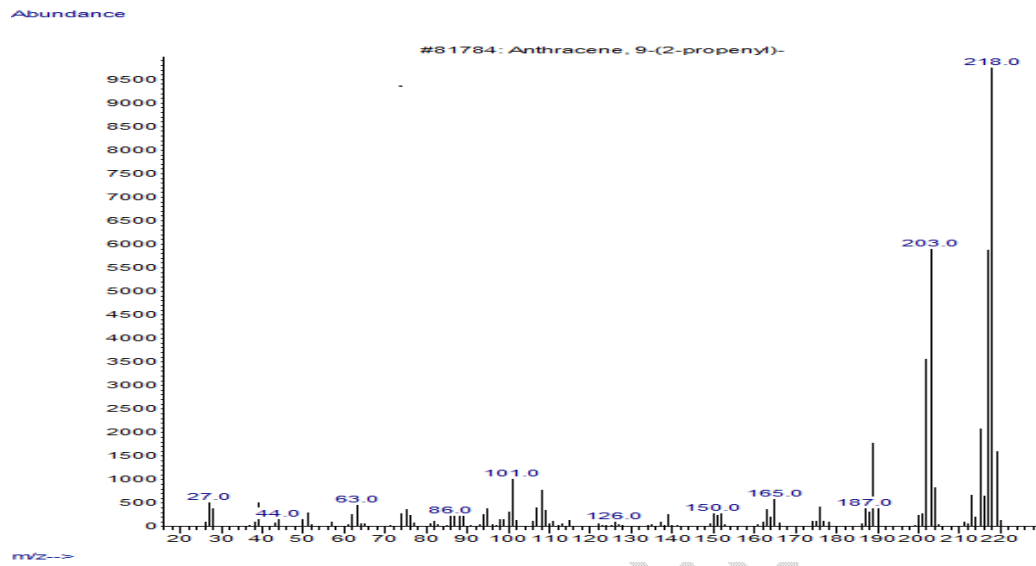


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Figure 3: shows the mass spectra of 4H-Pyran-3- carboxylic acid, 2-anono-5-cyano-6-ethyl-4-(3- pyridinyl)-methylester

UNDER PEER REVIEW

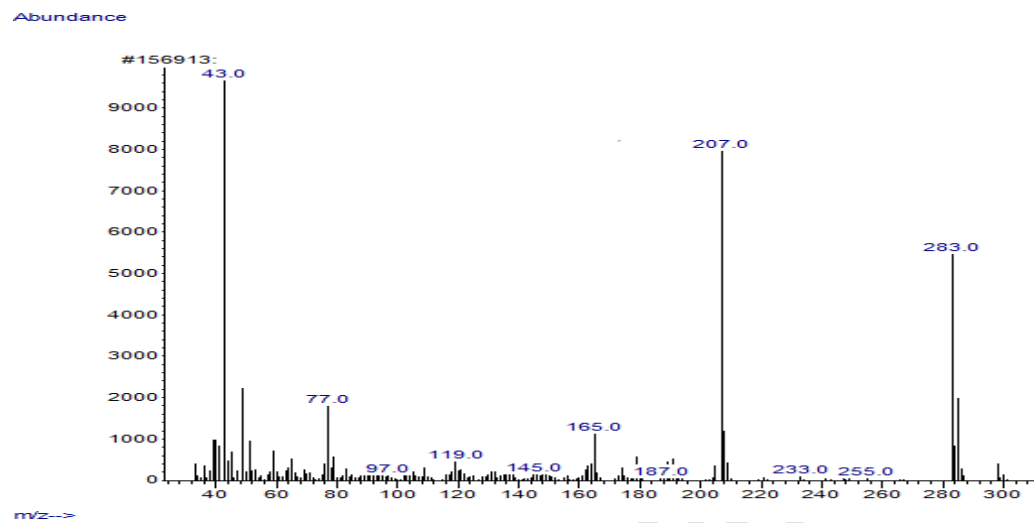


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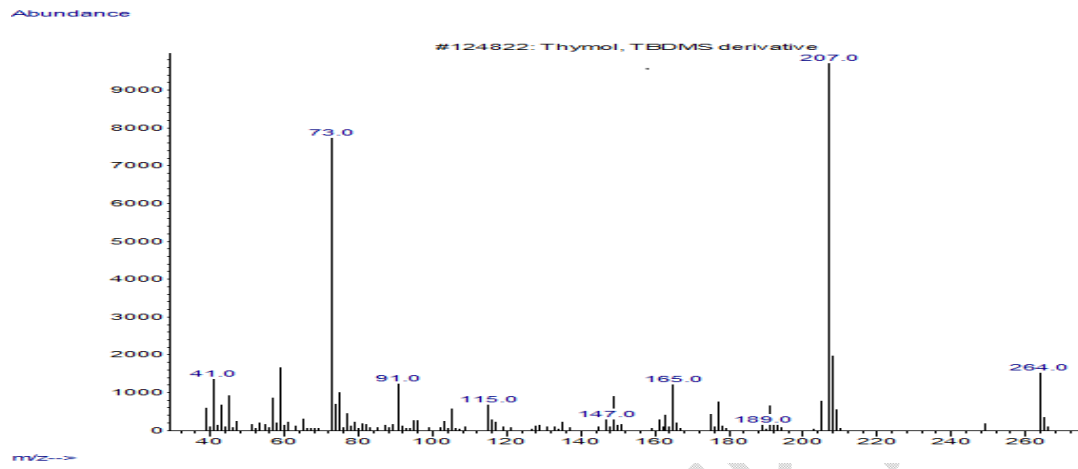
Figure 4: shows the mass spectra of Anthracene, 9-(2-propenyl)



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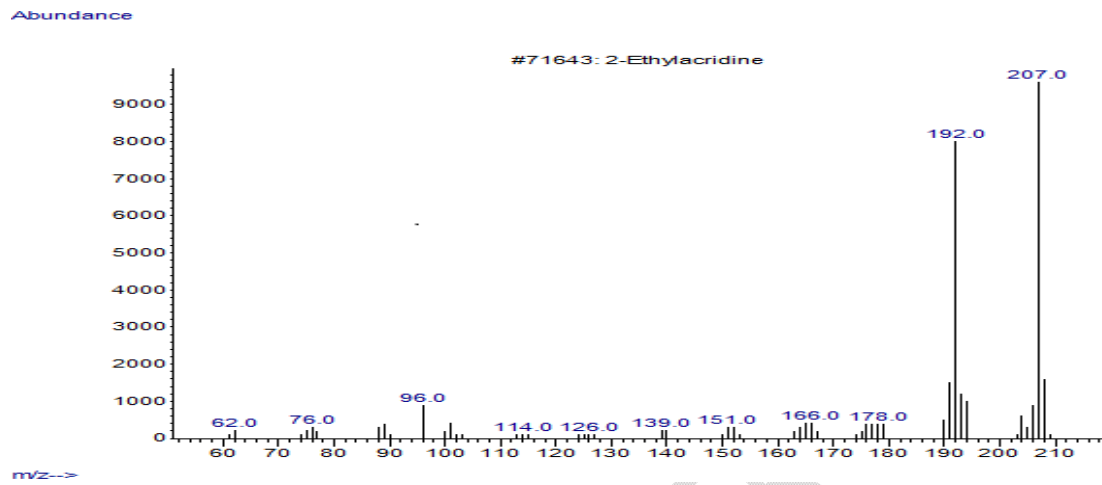
Figure 5: shows the mass spectra of 2,6-Lutidine 3,5-dichloro-4-dideylthio



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128 | **Figure 6: shows the mass spectra of Thymol, TBDS derivative**

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131 | **Figure 7: shows the mass spectra of 2-Ethylacridine**

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138 **DISCUSSION**

139 The study of plant-derived organic compounds and their activity has grown in recent years. Gas chromatography-mass spectrometry
140 (GC-MS) is a useful technique for identifying bioactive compounds with high accuracy [13].⁽⁴³⁾ In this present study, seventy-eight
141 bioactive compounds were identified from the aqueous extract of *Moringa oleifera* by GC-MS analysis. Some of the constituents are
142 identified in this plant for the first time such as ~~H~~-Indene, 2-butyl-5-hexyloctahydro, Anthracene, Cyclonoene (0.29%), d-lyxose
143 (0.22%), Cycloecosane(0.37%) and Democolcine (1.41%). *Moringa oleifera* plant has~~ve~~ been claimed to be utilized in phytomedicine
144 as an anti-inflammatory, anti-diabetic, anti-ulcer, antioxidant and hypocholesteromic substance [14].⁽⁴⁴⁾ From the GC-MS result,
145 *Moringa oleifera* contains Cis-Octadecenoic acid (cis-oleic acid) (C₁₈H₃₄O₂) which has a hypolipidemic activity which may be due to
146 the presence of a 5-alpha reductase inhibitor, which may have inhibited HMG-CoA reductase, a crucial enzyme in the biosynthesis of
147 cholesterol⁽⁸⁾. Furthermore, it has been shown that it can modulate some of the functions of neutrophils, thereby influencing the
148 inflammatory process. It prevents the function of protein kinase C in lymphocytes, the release of myeloperoxidase, and the chemotaxis
149 of human neutrophils⁽¹⁵⁾. Igwe *et al.* [8] report (2015)⁽⁶⁾ on GC/MS analysis of *Moringa oleifera* leaf extract also revealed that cis-
150 octadecenoic acid (19.16%) makes up a significant component of the leaf extracts. Anthracene, and 9-(2-propenyl) are known to have
151 significant biological activities against L1210 *in vitro* tumor cells⁽¹⁶⁾ Thymol, TBDMS derivative has been employed in conventional
152 medicine and demonstrated to have a variety of pharmacological actions which includes antioxidant⁽¹⁷⁾ free radical scavenging, anti-
153 inflammatory⁽¹⁸⁾ antibacterial⁽¹⁹⁾ analgesic⁽²⁰⁾ and antifungal⁽¹⁹⁾ activities. In addition, Thymol, TBDMS is routinely employed in
154 dentistry to treat oral cavity infections as a result of its potent antimicrobial properties⁽²¹⁾. 2,6- Lutidine 3,5- dichloro -4- dideylthio is

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155 | a natural heterocyclic aromatic organic compound found in nature. 2,6-Lutidine, 3,5-dichloro-4-did has been investigated for use as a
156 | food additive because of its nutty aroma⁽²²⁾. 4H-Pyran-3- carboxylic acid, 2-anono-5-cyano-6-ethyl-4-(3-pyridinyl)-methylester a rich
157 | source of biologically significant molecules is known to have a diverse range of biological and pharmacological processes such as
158 | antimicrobial, antiviral, cancer therapy and central nervous system activity⁽²³⁾ 2-Ethylacridine exhibits biological processes like anti-
159 | inflammatory anticancer, antimicrobial, antiparasitic, antimalarial, antiviral and fungicidal activities⁽²⁴⁾. These biological properties
160 | ~~are~~ as a result of their semi-planar heterocyclic structure, which interacts with a variety of biomolecular targets. Benzo(h)quinoline,
161 | 2,4-dimethyl has been reported to have bactericidal, DNA-binding, wound healing, and in vitro antioxidant activities ⁽²⁵⁾. Some
162 | important compounds identified as low level such as Octadecanoic acid (0.95%), 6-Octadecenoic acid(0.07%) and n-Hexadecanoic
163 | acid (1.97%) all have biological properties such as antibacterial, anti-inflammatory and antifungal activities. A similar study by
164 | Nishu *et al.*(2020)⁽²⁶⁾, Igwe *et al.*(2015)⁽⁸⁾ and Kingsley *et al.* (2021)⁽²⁷⁾ also identify these compounds in *Moringa oleifera* by GC-
165 | analysis. Myristoleic acid is a rare type of monounsaturated fatty acid ~~that~~ which is present in some edible human foods. It is known to
166 | induce apoptosis and exerts improvement on cardiovascular health parameters in humans⁽²⁸⁾ Demecolcine, identified for the first time
167 | is a natural alkaloid known for its antineoplastic activity which improves cancer radiotherapy outcomes by synchronizing tumor cells
168 | at metaphase, the radiosensitive stage of the cell cycle⁽²⁹⁾.

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170

171 **CONCLUSION**

172 *Moringa oleifera* leaf extract GC-MS analysis reveals a variety of bioactive compounds with both medicinal and therapeutic
173 potentials. The presence of these bioactive constituents in the leaves of *Moringa* provides a scientific foundation for its use in the
174 manufacturing of innovative drugs that would help in the treatment of various human infectious diseases. Furthermore, this study
175 provides a different strategy in antimicrobial therapy especially with antibiotic resistance on the rise.

176 **LIST OF ABBREVIATIONS**

177 GC MS: Gas chromatography–mass Spectrometry.

178 MW: Molecular weight

179 RT: Retention time

180

181 **DECLARATIONS DECLARATIONS**

182

183 **Availability of data and materials**

184 All data and materials are available upon request.

185

186 **Ethics approval and consent to participate**

187 Not applicable

188 **Consent for publication**

189 Not Applicable

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193 **REFERENCES**

- 194 1. Walter A, Samuel W, Peter A, Joseph O (2011). Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol
195 and n-hexane seed extracts on bacteria implicated in waterborne diseases. African Journal of Microbiology Research 5(2), 153-
196 157. <https://doi.org/10.5897/AJMR10.457>
- 197 2. Patel P, Nivedita P, Dhara P, Sharav D, Dhananjay, M (2014). Phytochemical analysis and antifungal activity of *Moringa*
198 *oleifera*. International Journal of Pharmacy and Pharmaceutical Sciences 6(5) 144-147.
199 [https://www.researchgate.net/profile/Sharav-](https://www.researchgate.net/profile/Sharav-Desai/publication/264540816_Phytochemical_analysis_and_antifungal_activity_of_moringa_oleifera/links/558ade6b08ae31beb1003700/Phytochemical-analysis-and-antifungal-activity-of-moringa-oleifera.pdf)
200 [Desai/publication/264540816_Phytochemical_analysis_and_antifungal_activity_of_moringa_oleifera/links/558ade6b08ae31be](https://www.researchgate.net/profile/Sharav-Desai/publication/264540816_Phytochemical_analysis_and_antifungal_activity_of_moringa_oleifera/links/558ade6b08ae31beb1003700/Phytochemical-analysis-and-antifungal-activity-of-moringa-oleifera.pdf)
201 [b1003700/Phytochemical-analysis-and-antifungal-activity-of-moringa-oleifera.pdf](https://www.researchgate.net/profile/Sharav-Desai/publication/264540816_Phytochemical_analysis_and_antifungal_activity_of_moringa_oleifera/links/558ade6b08ae31beb1003700/Phytochemical-analysis-and-antifungal-activity-of-moringa-oleifera.pdf)

- 202 3. Thilza, IB, Sanni S, Isah ZA, Sanni FS, Talle M, Joseph MB (2010). In vitro Antimicrobial activity of water extract of
203 *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases. *Academia Arena* 2(6), 80-82.
204 https://www.academia.edu/download/35412172/13_2681_moringa_aa0206_80_82.pdf
- 205 4. Pretha A, Ajaikuma BK, Robert AN, Bharat BA (2007). Bioavailability of curcumin: problems and promises. *Molecular*
206 *Pharmaceutics*, 4(6), 807–818. DOI: [10.1021/mp700113r](https://doi.org/10.1021/mp700113r)
- 207
- 208 5. Gustavo HFV, Jozeanne AM, Angela MA, Renata AC, Regine, HSDFV (2010). Antibacterial effect (in vitro) of *Moringa*
209 *oleifera* and *Annona muricata* against Gram-positive and Gram-negative bacteria. *Rev. Inst. Med. Trop. Sao Paulo*, 52(3), 129-
210 132. DOI: [10.1590/s0036-46652010000300003](https://doi.org/10.1590/s0036-46652010000300003)
- 211 6. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH (1994). Isolation and structure elucidation of new nitrile and
212 mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. *Journal of Natural Products* 57(9), 1256-
213 61. DOI: [10.1021/mp50111a011](https://doi.org/10.1021/mp50111a011)
- 214 7. Lawrence OAM, Peter L (2007). Phenolics of *Moringa oleifera* leaves. *Journal of Natural Product Research* 21(1), 56-
215 68. DOI: [10.1080/14786410601035811](https://doi.org/10.1080/14786410601035811)
- 216 8. Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku FJ (2015) GCMS analysis of Phytocomponents in the Methanolic
217 Extract of *Moringa oleifera* Leave. *Journal of Research in Pharmaceutical Science* 2(11), 1-6.
218 [https://www.researchgate.net/profile/Solomon-](https://www.researchgate.net/profile/Solomon-Ijioma/publication/308020922_GCMS_analysis_of_Phytocomponents_in_the_Methanolic_Extract_of_Moringa_oleifera_Leav)
219 [Ijioma/publication/308020922_GCMS_analysis_of_Phytocomponents_in_the_Methanolic_Extract_of_Moringa_oleifera_Leav](https://www.researchgate.net/profile/Solomon-Ijioma/publication/308020922_GCMS_analysis_of_Phytocomponents_in_the_Methanolic_Extract_of_Moringa_oleifera_Leav)

- 220 e/links/5809edc108ae1cd5f576b5ba/GCMS-analysis-of-Phytocomponents-in-the-Methanolic-Extract-of-Moringa-oleifera-
221 Leave.pdf
- 222 9. Nitesh B, Nitin I, Srilakshmi VP, Haranath C (2021). Phytochemical analysis of *Moringa oleifera* leaves extracts by GC-MS
223 and free radical scavenging potency for industrial applications. Saudi Journal of Biological sciences 28(12), 6915-6928.
224 <https://doi.org/10.1016/j.sjbs.2021.07.075>
- 225 10. Raheela J, Muhammad S, Amer J, Muhammad A (2008). Microscopic evaluation of the antimicrobial activity of seed extracts
226 of *Moringa oleifera*. Pakistan Journal of Botany 40(4), 1349- 1358. [http://pakbs.org/pjbot/PDFs/40\(4\)/PJB40\(4\)1349.pdf](http://pakbs.org/pjbot/PDFs/40(4)/PJB40(4)1349.pdf)
- 227 11. Jensen WB (2007). The origin of Soxhlex Extraction. Journal of Clinical Education 84 (12), 1913-1914.
228 <https://doi.org/10.1021/ed084p1913>
- 229 12. Buss AD, Butler MS (Eds.) (2010). Natural product chemistry for drug discovery, The Royal Society of Chemistry,
230 Cambridge p. 153
- 231 13. Mariswamy Y, Edward GW, Johnson M (2011). Chromatographic finger print analysis of steroids in Aervalanasa L. by
232 HPTLC technique. Asian Pal. J. Trop. Biomedicine 1, 428-433. doi: [10.1016/S2221-1691\(11\)60094-4](https://doi.org/10.1016/S2221-1691(11)60094-4)
- 233 14. Enas JK, Duha AA (2014). Phytochemical Characterization using GC-MS Analysis of Methanolic Extract of *Moringa oleifera*
234 (Family Moringaceae) Plant Cultivated in Iraq. Chemistry and Materials Research 6(5),pp, 9-26.
235 <https://core.ac.uk/download/pdf/234666473.pdf>

- 236 15. Hosana GR, Marco ARV, Juliana M, Haroldo F, Danielle MHC, Sandra HPF, Philip CC, Elaine H, Rui C (2010). Dietary Free
237 Oleic and Linoleic Acid Enhances Neutrophil Function and Modulates the Inflammatory Response in Rats. *Lipids* 45, pp. 809-
238 819. DOI: [10.1007/s11745-010-3461-9](https://doi.org/10.1007/s11745-010-3461-9)
- 239 16. Tanious FA, Jenkins TC, Neidle S, Wilson WD (1992). Substituent position dictates the intercalative DNA-binding mode for
240 anthracene-9,10-dione antitumor drugs. *Biochemistry* 31, 11632-11640. <https://doi.org/10.1021/bi00161a050>
- 241 17. Nedyalka VY, Emma MM, Michael HG, Violeta GR (1999). Antioxidant activity and mechanism of action of thymol and
242 carvacrol in two lipid systems. *Food Chemistry* 64, 59–66. [https://doi.org/10.1016/S0308-8146\(98\)00086-7](https://doi.org/10.1016/S0308-8146(98)00086-7)
- 243 18. Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J, Halliwell B, Aruoma OI (1994). Antioxidant actions of thymol,
244 carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem. Toxicol.* 32, 31–36. DOI: [10.1016/0278-6915\(84\)90033-4](https://doi.org/10.1016/0278-6915(84)90033-4)
- 245 19. Nicole D, Luc D, Madeleine P (1994). Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharm.*
246 *Acta Helv* 69, 25–28. [https://doi.org/10.1016/0031-6865\(94\)90027-2](https://doi.org/10.1016/0031-6865(94)90027-2)
- 247 20. Ozen T, Demirtas I, Aksit H (2011). Determination of antioxidant activities of various extracts and essential oil compositions
248 of *Thymus praecox* subsp. *skorpilii* var. *skorpilii*. *Food Chemistry* 124, 58–64. <https://doi.org/10.1016/j.foodchem.2010.05.103>
- 249 21. Shams TK, Merajuddin K, Javed A, Rizwan W, Omar HK, Javed M, Hamad ZA, Abdulaziz AA (2017). Thymol and
250 carvacrol induce autolysis, stress, and growth inhibition and reduce the biofilm formation by *Streptococcus mutans*. *AMB*
251 *Express* 7, 49. <https://doi.org/10.1186/s13568-017-0344-y>

- 252 22. Prudhomme DR, Park M, Wang Z, Buck JR, Rizzo, Carmelo J. (2000). Synthesis of 2'-Deoxyribonucleosides: B-3', 5'-Di-o-
253 benzoylthymidine. *Org. Synth.* **77**: 162. <https://doi.org/10.15227%2Forgsyn.077.0162>
- 254 **23.** David RA, Shridhar H, Emily R, Leslie G, William FV, Len L, Shaung L, Aruna S, Patricia AS, Laiqat M (2005).
255 Aminocyanopyridine inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2) Bioorg.
256 Med.Chem.Lett 15, 1587. <https://doi.org/10.1016/j.bmcl.2005.01.067>
- 257 **24.** Tripathi RP, Verma SS, Pandey J, Agarwal KC, Chaturvedi V, Manju YK, Srivastva AK, Gaikwad A, Sinha S.
258 (2006)Search of antitubercular activities in tetrahydroacridines: Synthesis and biological evaluation. *Bioorg. Med. Chem. Lett*
259 16, 5144-5147. <https://doi.org/10.1016/j.bmcl.2006.07.025>
- 260 25. Halehatty RPN, Halehatty SBN, Thangali RRN, Raja NH, Guothamchandra K, Riaz M, Khadeer ABM (2009). Synthesis of
261 novel benzo[h]quinolines: Wound healing, antibacterial, DNA binding and in vitro antioxidant activity. *European Journal of*
262 *Medicinal Chemistry*, 44(3):981-989. <https://doi.org/10.1016/j.ejmech.2008.07.006>
- 263 26. Nishu, Chandrawati J, Ravi K. (2020). Gas Chromatography Study of Methanolic Leave Extract of *Moringa oleifera* Lam.
264 *International Journal of Current Microbiology and Applied Sciences*, 9(2), 2590-2595.
265 <https://doi.org/10.20546/ijcmas.2020.902.295>
- 266 27. Enerijiofi KE, Akapo FH, Erhabor JO (2021). GC-MS analysis and antibacterial activities of *Moringa oleifera* leaf extracts on
267 selected clinical bacterial isolates. *Bulletin of the National Research Centre*, 45(179), 2-10.DOI:
268 <https://doi.org/10.1186/s42269-021-00640-9>

- 269 28. Iguchi K, Okumura N, Usui S, Sajiki H, Hirota K, Hirano K (2001). "Myristoleic acid, a cytotoxic component in the extract
270 from *Serenoa repens*, induces apoptosis and necrosis in human prostatic LNCaP cells". *The Prostate*. 47 (1): 59–65
271 <https://doi.org/10.1002/pros.1047>
- 272 29. Sutton M. (2005). Superior Mediastinal Obstruction Treated with Demecolcine Followed by Radiotherapy. *British Medical*
273 *Journal* 1(5433): 495–496. doi: [10.1136/bmj.1.5433.495](https://doi.org/10.1136/bmj.1.5433.495)
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