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

ABSTRACT

Background

The Evaluation of plants for its bioactive compounds is of great importance to researchers because of their therapeutic properties which can be harnessed to serve as alternatives to antibiotics or the development of novel drugs to fight human infectious diseases. *Moringa oleifera* is a member of the Moringaceae family with nutritional and medicinal properties.

Aim:

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

Methodology:

The bioactive compounds were extracted using the Soxhlet method of extraction and was analyzed using Gas chromatography mass-spectrometry.

Results

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

Conclusions

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

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

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] reported that *Moringa oleifera* leaf ethanolic extract contains niazirin, niazirinin, and niazininins A and B. Manguro & Lemmen, (2007) [7] also reported that benzoic acid, gallic acid, and beta benzaldehyde were isolated from a methanolic extract of *Moringa oleifera* leaves. Igwe *et al*. (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*. (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 al*. (2008) [10] revealed that seeds and leaves extracts show activity against different species of fungi such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Epidermophyton floccosum* and so on. Some of them are purely anthropophilic dermatophytes. These extracts have bactericidal and/or bacteriostatic activity against *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumoniae*

V. parahaemolyticus, *Enterococcus faecalis,Salmonella enteritidis,Aeromonas caviae, Pasteurella multocida, Bacillus subtilis, E. coli, Pseudomonas aeruginosa* [10]. In orderto utilize and make the best use of the available natural wealth, phytochemical screening is critical in identifying new sources of therapeutically and industrially valuable compounds with medicinal significance. As a result, the purpose of this study is to determine the phytochemical constituents of *Moringa oleifera* leaf extracts using GC–MS.

2.0 MATERIALS AND METHODS

2.1 Collection of plant materials

Fresh *Moringa Oleifera leaves* were taken from a mature healthy tree in a farmhouse at Owerri, Imo state, Nigeria. The leaves were identified and authenticated by Professor Cyril Duru of Crop science and Technology Department, Federal University of Technology, Owerri, Imo state, Nigeria. The plant was deposited in the herbarium of Department of Crop science and technology, Federal university of Technology, Owerri, with the voucher number CSTM/FUTO/4451.

2.2 Extraction of plant materials

Moinga oleifera leaves were firstly washed with sterilized water to remove dirt and debris. Jensen's Soxhlet method was used to 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

Celsius. After that, it was placed inside a desiccator and allowed to cool. 100g of the sample was weighed and poured into the soxhlet timble. To aid in extract filtering, the extraction thimble was lightly plugged with cotton wool, and the boiling flask was filled with 300ml of ethanol. The soxhlet device was put together and allowed to reflux at 600 degrees Celsius for 4 hours. After carefully removing the thimble, a volumetric flask was filled with the extracts and allowed to cool. The volumetric flasks contents were transferred to a rotatory evaporator in order to separate the solvent from the oil.

2.3 Extraction of phytochemicals

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 test tube was given 90 minutes to react. The reaction product in the test tube was transferred to a separator funnel after the reaction period. The tube was thoroughly cleaned with 20ml of ethanol, 10ml of cold water, 10ml of hot water, and 3ml of hexane before being moved into the funnel. The leaves extract was mixed with and washed three times with a 10ml aqueous solution of 10% v/v ethanol. After being dried with anhydrous sodium sulfate, the solvent was evaporated. The sample was dissolved in 1000ul of pyridine, with 200ul transferred into the vial for analysis.

2.4 Gas Chromatography- Mass spectrometry (GC-MS)

The GC–MS analysis of bioactive constituents from *Moringa Oleifera* leaf extracts was carried out using Agilent Technologies GC systems with the GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with the HP-5MS column (30 m in length 250 m in diameter 0.25 m in film thickness). GC–MS spectroscopic detection required a method for ionizing electrons

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 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 raised to 300 °C at 10 °C each minute. In a splitless mode, one microliter of the prepared 1% extracts diluted with respective solvents was injected. Based on the peak area produced in the chromatogram, the relative quantity of chemical compounds present in the extracts was expressed as a percentage.

2.5 Identification of bioactive constituents

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

3.0 RESULTS

The GC-MS analysis of the aqueous fraction of *Moringa oleifera* leaves extract revealed 78 peaks, which depicts the presence of 78 bioactive compounds. The chromatogram is shown in Figure 1 while the bioactive compounds with their retention time (RT), peak areas (%) molecular formula and molecular weight (MW) are shown in Table 1. The mass spectra of a few of the compounds with high concentrations are shown in Figure 2-7. Among the seventy-eight compounds identified, The main abundant compounds present

in *Moringa oleifera* were 1H-Indene, 2-butyl-5-hexyloctahydro (25.02%), 4H-Pyran-3- carboxylic acid, 2-anono-5-cyano-6-ethyl-4-(3- pyridinyl)-methylester (9.82%), Anthracene, 9-(2-propenyl) (6.72%), 2,6- Lutidine 3,5- dichloro -4- dideiylthio (5.67%), 1-Hydroxy-3- methoxypropan -2- yl oleate (5.19%), Thymol,TBDMS derivative (5.02%), 2- Ethylacridine (3.28)%, 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 – Benzenediol, 3,5- bis(1,1-dimethylethyl) (2.63), Propanamide, N(3- methoxyphenyl) -2,2- dinethyl (2.59%), Cis-13-Octadecenoic 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-methyl-5- oxo-3 phenylthio) (2.11%) and various other compounds were identified as low level.

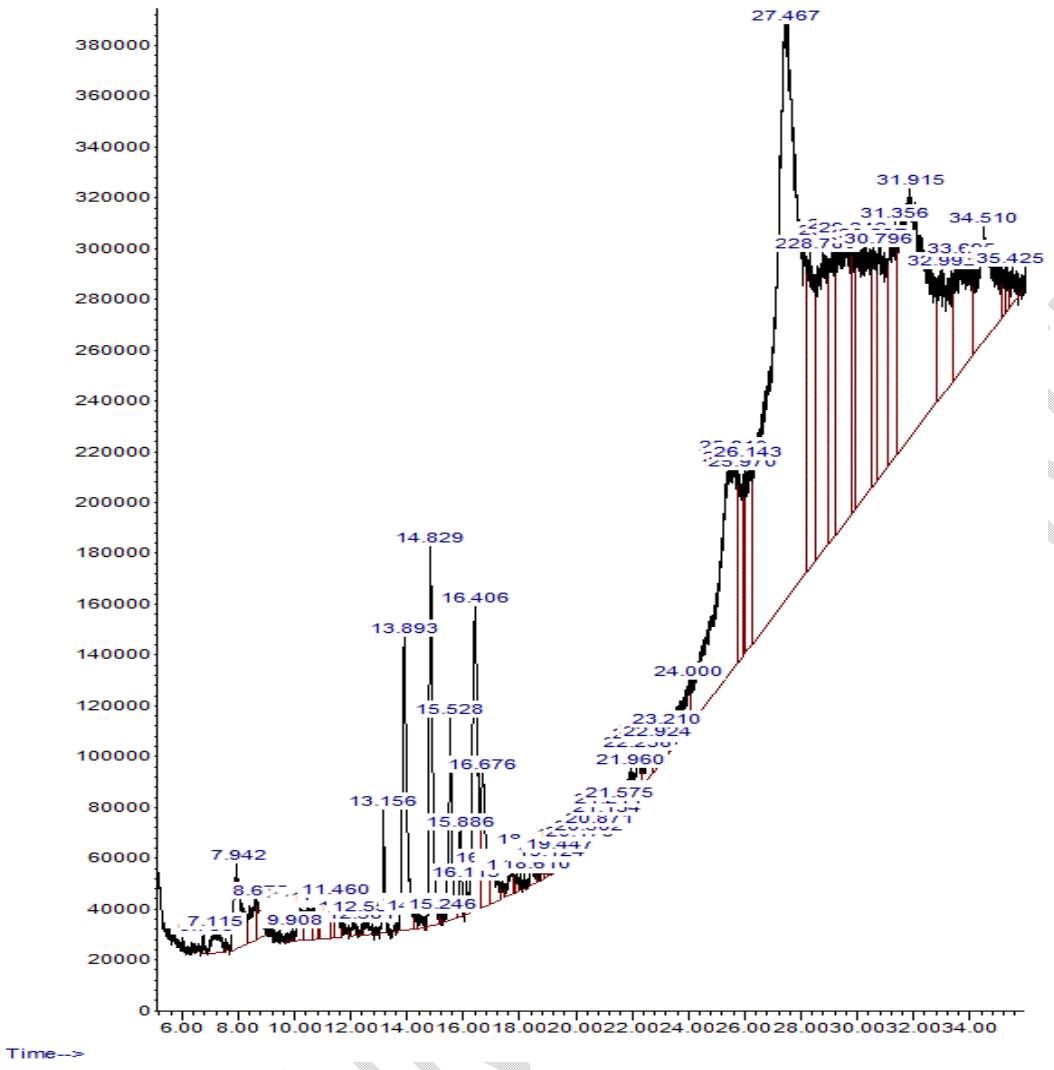

FIGURE 1: shows GC-MS chromatogram of aqueous extract of *Moringa oleifera*

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

S.NO	Name of Compound	RT (retention time)	Peak area	Molecular formula	MW
1	2-Fluorobenzyl alcohol	6-555	0.05	$C_7H_7F_0$	126
2	(IS, 2R, 4R, 7R) -4-Iso propyl-7-methyl -3,8-dioxatricyclo (5.1.0.02, 4) octane	7.115	0.29	$C_{10}H_{16}O_2$	168
3	1-Nitro-2-acetamodo-1, 2-dodeoxy-d-mannitol	7.942	0.95	$C_8H_{16}N_2O_7$	252
4	Butanoic acid, 3–hydroxyl-, ethylester	8.586	0.34	$C_6H_{12}O_3$	132
5	2H- Azepin-2-acetamodo-1, 2-dodeoxy-d-mannitol	7.942	0.95	$C_6H_{12}O_3$	132
6	6-Acetyl-beta-d-mannose	9.908	0.05	$C_8H_{14}O_7$	222
7	Undec-10-ynoic acid, undecyl ester	10.206	0.28	$C_{22}H_{140}O_2$	336
8	Cyclononene	10.466	0.29	C_9H_{16}	124
9	Dodecyl proylether	10.705	0.18	$C_{15}H_{32}O$	228
10	Decanoic acid, 2,3-dihydroxypropylester	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	$C_5H_{22}O_{11}$	3
13	d-lyxose	11.460	0.22	C_5H100S	150
14	Undec-10-ynoic acid, tetradecyl esther	12.076	0.09	$C_{25}H_{46}O_2$	378
15	Cydopentadecanone, 2-hydroxy	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-Octadecadie n-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	9-octadecenal	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	11- Octadecenoic acid, methyl esther	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	$C_{13}H_{21}CL_3O_2$	315
27	Cis-13-Octadecenoic acid	16.406	2.46	$C_{18}H_{34}O_2$	282
28	Octadececanoic 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-Dodecen1-ol trifluoroacetate	17.714	0.16	$C_{14}H_{23}F_3O_2$	280
31	(z)-9-octadecen 1-olide	17.768	0.03	$C_{18}H_{32}O_2$	280
32	1,3,12-Nonadecatriene	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	$C_3H_3F_3O_2$	128
39	9- Octadecenoic acid (z), -2hydroxyl-1-	19363	0.06	$C_{21}H_{38}O_4$	354

	(hydroxymethyl) ethylester				
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	Octadadec -9- enoicacid	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) – Ethyloleate	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	$C_6H_5CL_3O_3$	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	$C_{16}H_{48}O_7S_{18}$	577
52	Octadec-9-enoic acid	22.238	0.33	$C_{18}H_{34}O_2$	282
53	Propyleneglycol monoleate	22.536	0.36	$C_{21}H_{40}O_3$	340
54	9- octadecenoic acid(z), 2-hydroxy-1-1(hydroxyl methyl) ethyjester	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-chloro undecanoate	23.210	0.21	$C_{12}H_{22}C_{12}O_2$	269
57	Acetamode, 2-chloro N-(3-cyano – 4,6- dihydro-4,4,6,6-tetramethylthieno (2,3-c) furan-2yl)	24.000	0.82	$C_{19}H_{13}CIN_2O_2$	336

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	$C_{13}H_{10}F_3O_2\,N_3O$	281
61	2,6-lutidone, 3,5-dichloro-4-cyclohexylthio	26.143	1.35	$C_{19}H_{31}CL_{12}O_2N_S$	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	$C_{13}H_{22}N_5Si$	222
65	3-pyridinamine, 2- (4-methyl -4H-1, 2-4- triazol-3-yl) thio]	29.170	2.28	$C_8H_9N_5S$	207
66	2,6-Lutidine 3,5-dichloro-4-dodecylthio	29.470	5.67	$C_{19}H_{31}CL_2NS$	376
67	2-(n-propyl) oxgben zylidene acetophenone acetophenone	29.843	1.72	$C_{11}H_{14}O$	162
68	1- Hydroxy-3-methoxypropan -2- yl oleate	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	$C_{17}H_{15}CIN_4O_2$	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
73	Cyclopenteno(4,3-b) tetrahydrofuran, 3-[(4-methyl-5-	32.992	2.11	$C_{19}H_{18}O_5S$	358

	oxo-3 phenylthio)				
74	Propanamide, N(3- methoxyphenyl) -2,2- dinethyl	33.695	2.59	$C_{12}H_{17}NO_2$	207
75	1,2 – Benzenediol, 3,5- bis (1,1-dimethylethyl)	34.510	2.63	$C_{14}H_{22}O_2$	222
76	1- Methyl-4-Phnenyl-5-Thioxo-1,2,4- Triazolodon-3-One	35.168	0.19	$C_9H_9N_3OS$	207
77	phenol, 6-methyl-2-[(4-morpholonyl)methyl]	35.312	0.17	$C_{12}H_{17}NO_2$	207
78	1H-ihdole, 5-methyl-2- phenyl	35.425	0.28	$C_{15}H_{13}N$	207

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

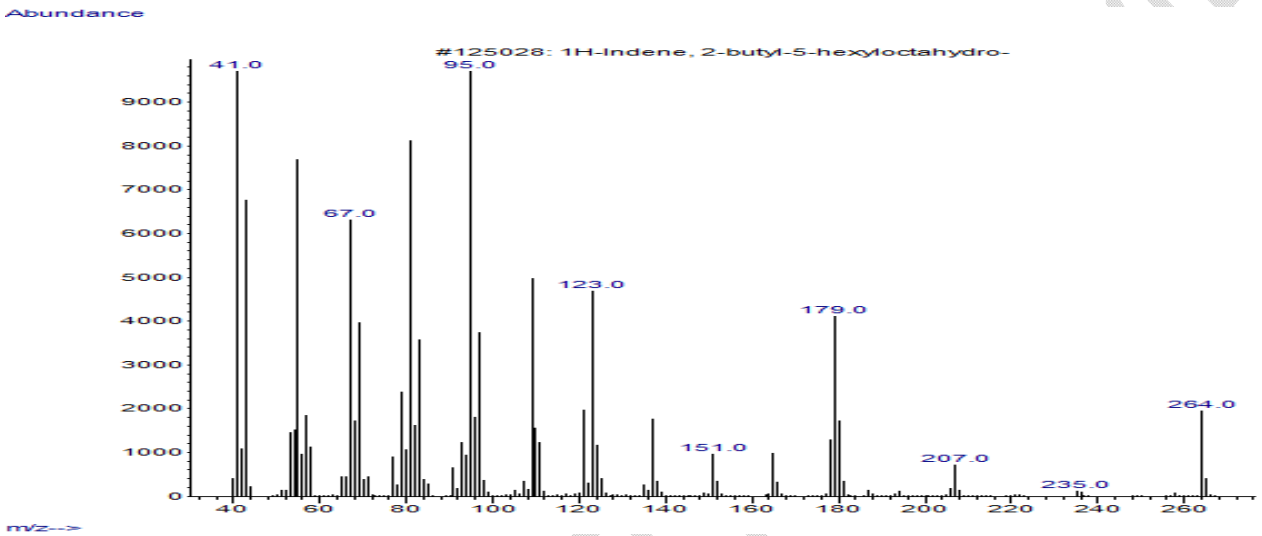

Figure 2: shows the mass spectra of 1H-Indene, 2-butyl-5-hexaloctahydro

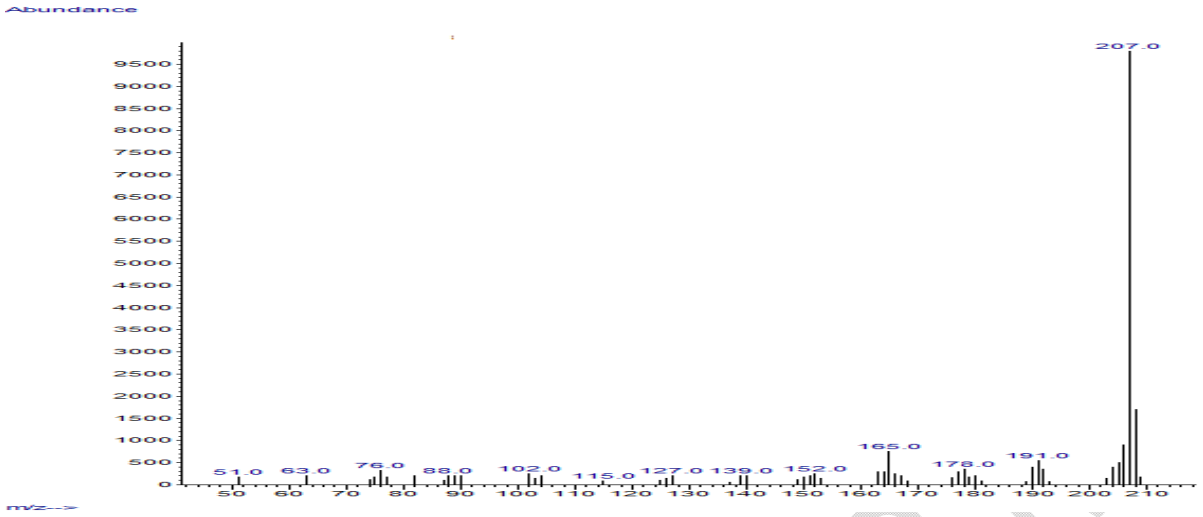

Figure 3: shows the mass spectra of 4H-Pyran-3- carboxylic acid, 2-anono-5-cyano-6-ethyl-4-(3- pyridinyl)-methylester

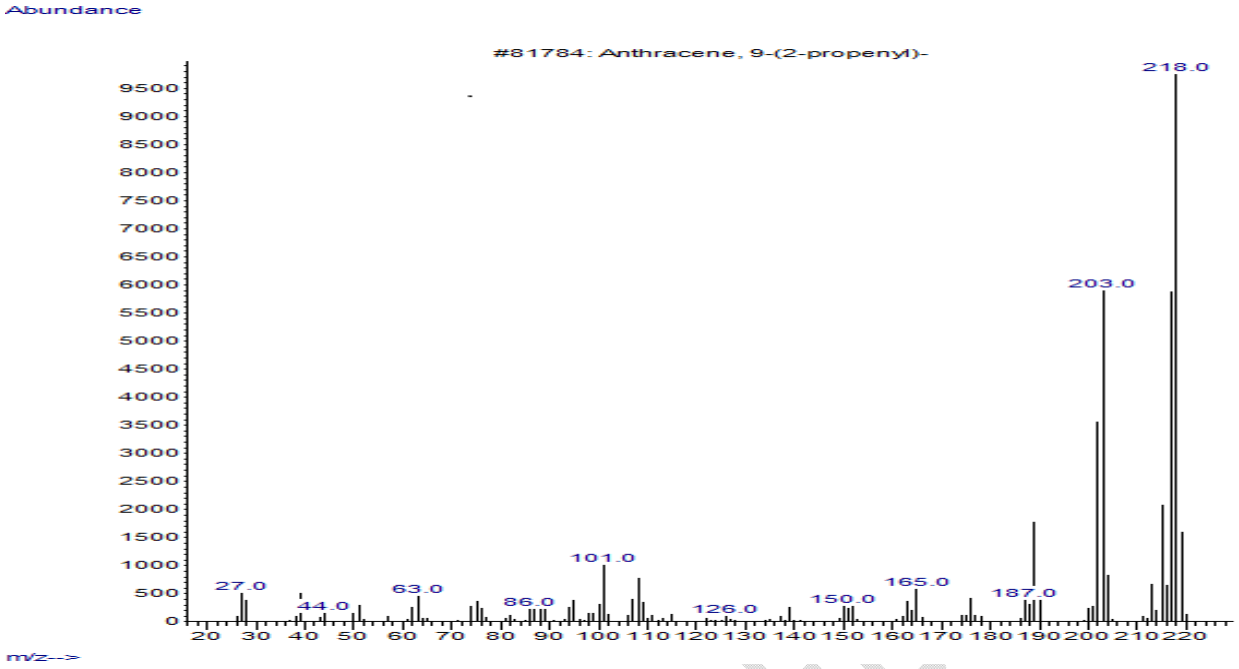

Figure 4: shows the mass spectra of Anthracene, 9-(2-propenyl)

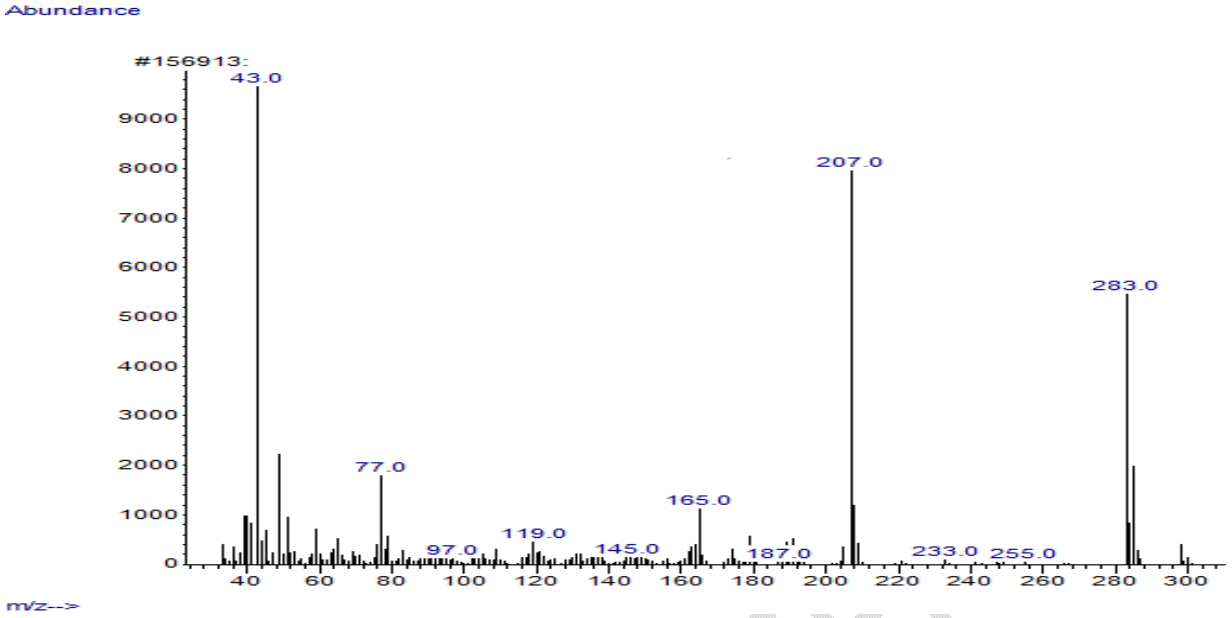

Figure 5: shows the mass spectra of 2,6- Lutidine 3,5- dichloro -4- dideiylthio

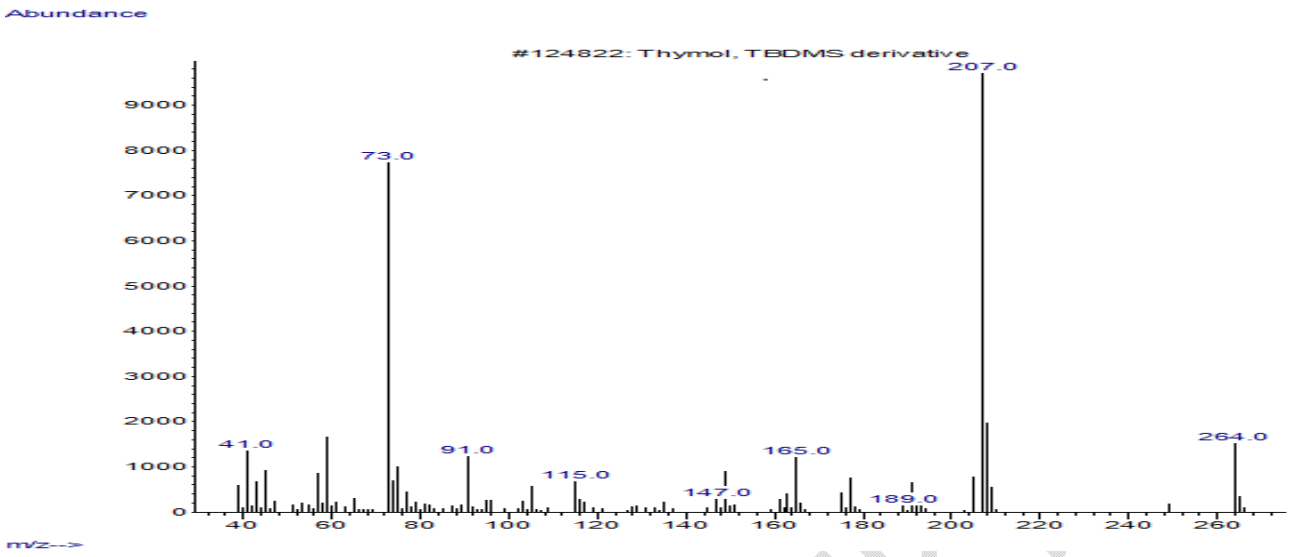

Figure 6: shows the mass spectra of Thymol, TBDS derivative

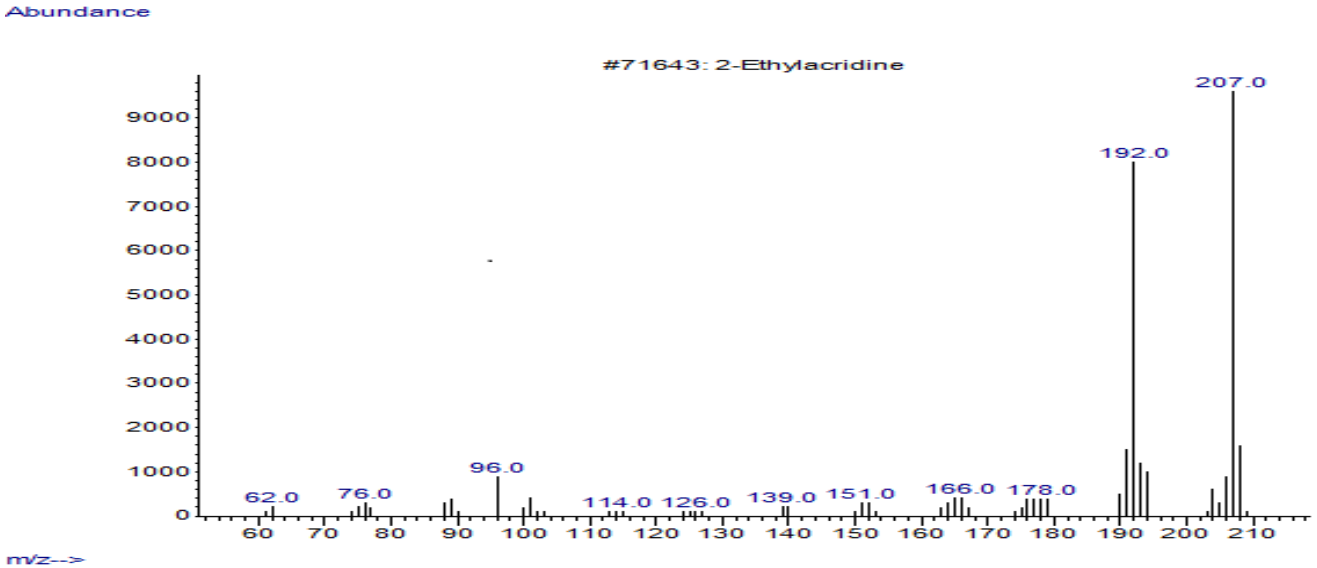

Figure 7: shows the mass spectra of 2- Ethylacridine

4.0 DISCUSSION

The study of plant-derived organic compounds and their activities has grown in recent years. Gas chromatography-mass spectrometry (GC-MS) is a useful technique for identifying bioactive compounds with high accuracy [13]. In this present study, seventy-eight bioactive compounds were identified from the aqueous extract of *Moringa oleifera* by GC-MS analysis. Some of the constituents are identified in this plant for the first time such as 1H-Indene, 2-butyl-5-hexyloctahydro, Anthracene, Cyclonoene (0.29%), d-lyxose (0.22%), Cycloeicosane(0.37%) and Democolcine (1.41%). *Moringa oleifera* plant have been claimed to be utilized in phytomedicine asan anti-inflammatory, anti-diabetic, anti-ulcer, antioxidantand hypocholesteromic substance [14]. From the GC-MS result, *Moringa oleifera* contains Cis-Octadecenoic acid (cis-oleic acid) ($C_{18}H_{34}0_2$) which has a hypolipidemic activity which may be due to the presence of a 5-alpha reductase inhibitor, which may have inhibited HMG-CoA reductase, a crucial enzyme in the biosynthesis of cholesterol [8]. Furthermore, it has been shown that it can modulate some of the functions of neutrophils, thereby influencing the inflammatory process. It prevents the function of protein kinase C in lymphocytes, the release of myeloperoxidase, and the chemotaxis of human neutrophils [15]. Igwe *et al*. (2015) [8] on GC/MS analysis of *Moringa oleifera* leaf extract also revealed that cis-octadecenoic acid (19.16%) makes up a significant component of the leaf extracts. Anthracene, 9-(2-propenyl) are known to have significant biological activities against L1210 *in vitro* tumor cells [16]. Thymol, TBDMS derivative has been employed in conventional medicine and demonstrated to have a variety of pharmacological actions which includes antioxidant, [17] free radical scavenging, anti-inflammatory, [18] antibacterial, [19] analgesic [20] and antifungal [19] activities. In addition, Thymol, TBDMS is routinely employed in dentistry to treat oral cavity infections as a result of its potent antimicrobial properties [21]. 2,6- Lutidine 3,5-

dichloro -4- dideiylthio is a natural heterocyclic aromatic organic compound found in nature.2,6-Lutidine, 3.5-dichloro-4-did has been investigated for use as a 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 source of biologically significant molecules is known to have a diverse range of biological and pharmacological processes such as antimicrobial, antiviral, cancer therapy and central nervous system activity[23]. 2-Ethylacridine exhibits biological processes like anti-inflammatory anticancer, antimicrobial, antiparasitic, antimalarial, antiviral and fungicidal activities[24]. These biological properties is as a result of their semi-planar heterocyclic structure, which interacts with a variety of biomolecular targets. Benzo(h)quionoline, 2,4-dimethylhas been reported to have bactericidal, DNA-binding, wound healing, and invitro antioxidant activities [25]. Some important compounds identified as low level such as Octadecanoic acid (0.95%), 6-Octadecenoic acid(0.07%) and n-Hexadecanoic acid(1.97%) all have biological properties such as antibacterial, anti-inflammatory and antifungal activities. Similar study by Nishu *et al*.(2020) [26], Igwe *et al*.(2015) [8] and Kingsley *et al*. (2021) [27] also identifies these compounds in *Moringa oleifera* by GC-analysis. Myristoleic acid is a rare type of monounsaturated fatty acid which is present in some edible human foods. It is known to induce apoptosis and exerts improvement on cardiovascular health parameters in humans [28]. Demecolcine, identified for the first time is a natural alkaloid known for its antineoplastic activity which improves cancer radiotherapy outcomes by synchronizing tumor cells at metaphase, the radiosensitive stage of the cell cycle [29].

5.0 CONCLUSION

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

LIST OF ABBREVIATIONS

GC MS: Gas chromatography–mass Spectrometry.

MW: Molecular weight

RT: Retention time

DECLEARATIONS

Availability of data and materials

All data and materials are available upon request.

REFRENCES

1. Walter A, Samuel W, Peter A, Joseph O (2011). Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts on bacteria implicated in waterborne diseases. African Journal of Microbiology Research 5(2), 153-157. https://doi.org/10.5897/AJMR10.457
2. Patel P, Nivedita P, Dhara P, Sharav D, Dhananjay, M (2014). Phytochemical analysis and antifungal activity of *Moringa oleifera.* International Journal of Pharmacy and Pharmaceutical Sciences 6(5) 144-147. 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
3. Thilza, IB, Sanni S, Isah ZA, Sanni FS, Talle M, Joseph MB (2010). In vitro Antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases. Academia Arena 2(6), 80-82. https://www.academia.edu/download/35412172/13_2681_moringa_aa0206_80_82.pdf
4. Pretha A, Ajaikuma BK, Robert AN, Bharat BA (2007). Bioavailability of curcumin: problems and promises. *Molecular Pharmaceutics,* 4(6), 807–818. DOI: 10.1021/mp700113r

5. Gustavo HFV, Jozeanne AM, Angela MA, Renata AC, Regine, HSDFV (2010). Antibacterial effect (in vitro) of *Moringa oleifera andAnnona muricata* against Gram-positive and Gram-negative bacteria. Rev. Inst. Med. Trop. Sao Paulo, 52(3), 129-132.DOI: 10.1590/s0036-46652010000300003
6. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. Journal of Natural Products 57(9), 1256-61.DOI: 10.1021/np50111a011
7. Lawrence OAM, Peter L (2007). Phenolics of *Moringa oleifera* leaves. Journal of Natural Product Research 21(1), 56-68.DOI: 10.1080/14786410601035811
8. Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku FJ (2015) GCMS analysis of Phytocomponents in the Methanolic Extract of Moringa oleifera Leave. Journal of Research in Pharmaceutical Science 2(11), 1-6. https://www.researchgate.net/profile/Solomon-Ijioma/publication/308020922_GCMS_analysis_of_Phytocomponents_in_the_Methanolic_Extract_of_Moringa_oleifera_Leave/links/5809edc108ae1cd5f576b5ba/GCMS-analysis-of-Phytocomponents-in-the-Methanolic-Extract-of-Moringa-oleifera-Leave.pdf
9. Nitesh B, Nitin I, Srilakshmi VP, Haranath C (2021). Phytochemical analysis of *Moringa oleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applications. Saudi Journal of Biological sciences 28(12), 6915-6928. https://doi.org/10.1016/j.sjbs.2021.07.075

10. Raheela J, Muhammad S, Amer J, Muhammad A (2008). Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pakistan Journal of Botany 40(4), 1349- 1358. http://pakbs.org/pjbot/PDFs/40(4)/PJB40(4)1349.pdf
11. Jensen WB (2007). The origin of Soxhlex Extraction. Journal of Clinical Education 84 (12), 1913-1914. https://doi.org/10.1021/ed084p1913
12. Buss AD, Butler MS (Eds.) (2010). Natural product chemistry for drug discovery, The Royal Society of Chemistry, Cambridge p. 153
13. Mariswamy Y, Edward GW, Johnson M (2011). Chromatographic finger print analysis of steroids in Aervalanasa L. by HPTLC technique. Asian Pal. J. Trop. Biomedicine 1, 428-433. doi: 10.1016/S2221-1691(11)60094-4
14. Enas JK, Duha AA (2014). Phytochemical Characterization using GC-MS Analysis of Methanolic Extract of *Moringa oleifera* (Family Moringaceae) Plant Cultivated in Iraq. Chemistry and Materials Research 6(5),pp, 9-26. https://core.ac.uk/download/pdf/234666473.pdf
15. Hosana GR, Marco ARV, Juliana M, Haroldo F, Danielle MHC, Sandra HPF, Philip CC, Elaine H, Rui C (2010). Dietary Free Oleic and Linoleic Acid Enhances Neutrophil Function and Modulates the Inflammatory Response in Rats. Lipids 45, pp. 809-819.DOI: 10.1007/s11745-010-3461-9
16. Tanious FA, Jenkins TC, Neidle S, Wilson WD (1992). Substituent position dictates the intercalative DNA-binding mode for anthracene-9,10-dione antitumor drugs. Biochemistry 31, 11632-11640. https://doi.org/10.1021/bi00161a050

17. Nedyalka VY, Emma MM, Michael HG, Violeta GR (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem*istry 64, 59–66. https://doi.org/10.1016/S0308-8146(98)00086-7

18. Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J, Halliwell B, Aruoma OI (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxicol. 32, 31–36.DOI: 10.1016/0278-6915(84)90033-4

19. Nicole D, Luc D, Madeleine P (1994). Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharm. Acta Helv 69, 25–28. https://doi.org/10.1016/0031-6865(94)90027-2

20. Ozen T, Demirtas I, Aksit H (2011). Determination of antioxidant activities of various extracts and essential oil compositions of Thymus praecox subsp. skorpilii var. skorpilii. Food Chemistry 124, 58–64. https://doi.org/10.1016/j.foodchem.2010.05.103

21. Shams TK, Merajuddin K, Javed A, Rizwan W, Omar HK, Javed M, Hamad ZA, Abdulaziz AA (2017). Thymol and carvacrol induce autolysis, stress, and growth inhibition and reduce the biofilm formation by *Streptococcus mutans*. AMB Express 7, 49. https://doi.org/10.1186/s13568-017-0344-y

22. Prudhomme DR, Park M, Wang Z, Buck JR, Rizzo, Carmelo J. (2000). Synthesis of 2'-Deoxyribonucleosides: B-3', 5'-Di-o-benzoylthymidine. *Org. Synth.* **77**: 162. https://doi.org/10.15227%2Forgsyn.077.0162

23. David RA, Shridhar H, Emily R, Leslie G, William FV, Len L, Shaung L, Aruna S, Patricia AS, Laiqat M (2005). Aminocyanopyridine inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2) Bioorg. Med.Chem.Lett 15, 1587. https://doi.org/10.1016/j.bmcl.2005.01.067

24. Tripathi RP, Verma SS, Pandey J, Agarwal KC, Chaturvedi V, Manju YK, Srivastva AK, Gaikwad A, Sinha S. (2006)Search of antitubercular activities in tetrahydroacridines: Synthesis and biological evaluation. Bioorg. Med. Chem. Lett 16, 5144-5147. https://doi.org/10.1016/j.bmcl.2006.07.025

25. Halehatty RPN, Halehatty SBN, Thangali RRN, Raja NH, Guothamchandra K, Riaz M, Khadeer ABM (2009). Synthesis of novel benzo[h]quinolines: Wound healing, antibacterial, DNA binding and in vitro antioxidant activity. European Journal of Medicinal Chemistry, 44(3):981-989. https://doi.org/10.1016/j.ejmech.2008.07.006

26. Nishu, Chandrawati J, Ravi K. (2020). Gas Chromatography Study of Methanolic Leave Extract of *Moringa oleifera* Lam. International Journal of Current Microbiology and Applied Sciences, 9(2), 2590-2595. https://doi.org/10.20546/ijcmas.2020.902.295

27. Enerijiofi KE, Akapo FH, Erhabor JO (2021). GC–MS analysis and antibacterial activities of *Moringa oleifera* leaf extracts on selected clinical bacterial isolates. Bulletin of the National Research Centre, 45(179), 2-10.DOI: https://doi.org/10.1186/s42269-021-00640-9

28. Iguchi K, Okumura N, Usui S, Sajiki H, Hirota K, Hirano K (2001). "Myristoleic acid, a cytotoxic component in the extract from Serenoa repens, induces apoptosis and necrosis in human prostatic LNCaP cells". The Prostate. 47 (1): 59–65 https://doi.org/10.1002/pros.1047

29. Sutton M. (2005). Superior Mediastinal Obstruction Treated with Demecolcine Followed by Radiotherapy. British Medical Journal 1(5433): 495–496.doi: 10.1136/bmj.1.5433.495