Identification of Compounds in *Elaeis guineensis* Fruits using GC-MS

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ABSTRACT: The aim of this study was to identify the chemical constituents of *Elaeis guineensis* (EG) using GC-MS. EG fruits were purchased from a local market in Edo state and was identified, cleaned, boiled and extracted. The resultant crude extract was strained through filter paper and partitioned into n-hexane, dichloromethane and ethyl acetate before subjected to GC-MS analysis. Eighty-two (82) compounds were identified and the major components are hexadecanoic acid, methyl ester (10.53%), oleic acid (25.92%). n-hexadecanoic acid (31.41%), cis-vaccenic acid (22.82%) and octadecanoic acid (13.16%). Plants fatty acids are important for growth and defence mechanism against pathogen, possess antimicrobial action, implicated in pathway elucidation and stimulation of resistance mechanism in plants. Long chain fatty acids exert their effect on membrane phospholipids by modifying its composition and potentially interfering with synthesis of lipid signalling molecules. Identification of EG fruits have provided an idea of its chemical composition of the extract of *E. guineensis*.

Key words: *Elaeis guineensis* fruit, fatty acid, GC-MS and vaccenic acid.

INTRODUCTION

Elaeis guineensis (EG) known as African oil palm, is a perennial monocot plant of the family Arecaceae[1] and originate from the Gulf of Guinea in tropical rain forest of Africa.[2] It was first introduced to other countries in 15th century by the Portuguese explorer.[3] The palm fruit is an orange red drupe which produces 45-55% oil, from the fleshy mesocarp by mechanical milling or by traditional method. The colour of the oil palm ranges from light yellow to orange-red with melting point of 25^{o}C.[1,4]

It is used ethno-medicinally for the management and treatment of joint pain, headache and to enhance libido. It is used as diuretic, in the promotion of wound healing and to promote the expression of breast milk during lactation.[5,6] It is also used orally as poison antidote and externally with many other herbs as lotion for skin diseases. Basically, palm oil is use for cooking, soap making, creams and cosmetics production.[5]

Gas chromatography (GC) is a widely applied technique that plays fundamental role in detection of the components and their proportions in a mixture.[7]

When it is couple to a mass spectrometer (MS), it has the advantage of simultaneously determining the molecular weight of each component detected. The disadvantage with this method include interference and overlap from co-extracted sample, can be reduced or overcome by the selection of appropriate fragment ions and analytical columns.[8]

Literature search have not shown the profiling of EG fruit extract for its chemical constituent. Thus the need for the profiling of the fruit extract of EG, to obtain an idea of the compounds that responsible for some of its activities.

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MATERIALS AND METHODS

Plant collection and identification. The fruit of EG was purchased at Oba market, Oredo Local Government Area of Edo State, in September 2019. It was identified at the Department of Plant Biology and Biotechnology, University of Benin, by Dr. Akinnibosun H.A. and voucher specimen was deposited with a herbarium number of UBH-E444.

Preparation and extraction of the fruit. The fruits of EG fruits were hand-picked and washed with distilled water to remove dirt and other possible contaminants. About 1.5 kg of EG fruit was added to a 2 litres of boiling water at 100 ^{O}C and allowed to boil for about 45 minute, thereafter, the water was decanted and the boiled EG fruit was transferred into a mortar and a pestle was used to triturated before adding about 1litre of warm distilled water. Trituration was continued until the fleshy mesocarp was separated. About 500 millilitre of deionized water was added to the content and stirred continuously until all the fleshy components were completely removed from the seed to obtain the extract. The palm kernel seeds and chaft were filtered out from the crude extract. The content crude extract was further concentrated by passing it through the filter paper to remove the water content. The endocarp and fibrous mesocarp were discarded. The resultant crude extract was strained through a filter paper and the residue was weighed with the aid of a weighing scale and stored in a beaker in a refrigerator.

Partitioning. Fractionation was done by weighing about 189g of the concentrated crude extract into a beaker and dissolve with 100 ml of methanol before 250 ml of water. These were vigorously stirred before transferring to a separating funnel. About 200 ml of n-hexane was added to the mixture and rocked gently to prevent the formation of emulsion and the content was opened occasionally to expel out gases formed in the separating funnel. The mixture was left on standing for about 1hr before decanting the n-hexane fraction. Similar volume of n-hexane was added until the extract became colourless. Similar procedure was carried out by using dichloromethane and ethyl acetate solvents. Each of the samples so obtained were labelled appropriately and subjected to Gas chromatography mass spectroscopy (GC-MS) analysis

GC-MS condition. Gas chromatography- mass spectrometry (GC-MS) analyses of the fractions were performed on QP 2010 SE Shimadzu Japan. Separation was achieved on a restek Rt x 5ms column (0.32 film thickness). The GC operating conditions were as follows. Temperature holds at 90^{o}C for 1 min, increases from 90^{o}C to 150^{o}C at a rate of 13 min and with final isothermal hold at 300^{o}C at a rate of 13 min and with final isothermal hold at 300^{o}C for 2 min. helium was used as the carrier gas. The sample was injected in the split mode with the injector temperature at 250^{o}C. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 45 to 700 Dalton. Data were acquired and processed using ChemStation software. Compounds were identified by comparing their base peak and two other major peaks (selected ion monitoring mode) from the fragmentation pattern for each compound and these were compared with data from National Institute of Standard Technology (NIST) library.

RESULTS AND DISCUSSION

The retention time of a compound could be used as a means of qualitative identification, but that could mean matching with standards.[9] This could lead to overlap in the retention time in some case, which is surmounted by using MS as the detector. GC-MS analysis for compounds present in EG, was done in n-hexane, dichloromethane and ethyl acetate fractions. The result showed that twenty-nine (29) compounds were identified from ethyl acetate fraction, twenty-eight (28) from n-hexane fraction and twenty-five (25) from dichloromethane fraction. The peaks were individually identified by comparing the detected mass-to-charge (m/z) ratios against a standard mass spectrum from National Institute of Standards and Technologies 11 (NIST 11) Library with peaks similarity. This was aided by

identification of the molecular ion (M^+), base peak and two confirmatory ions.

Figures 1, 2 and 3 show the peaks from the chromatogram of the fractions (ethyl acetate, dichloromethane and n-hexane) of EG fruits. These peaks were identified by passing them through a mass spectrometer and the fragmentation pattern for each peak was matched with NIST library using selected ion mentoring mode.

Table 1 shows the level of different compounds from ethyl acetate fraction of EG, including hexadecanoic acid, methyl ester (10.53%), oleic acid (14.29%), 9-octadecenoic acid, methyl ester (E) (9.46%) and n-hexadecanoic acid (18.75%) which were observed to be high. High level of fatty acids and esters in EG is in agreement with previous study.[10] The presence of hexadecanoic acid is an important growth and defence mechanism against pathogen in EG.[11] Fatty acids have been shown to possess antimicrobial action;[12] while others have been implicated in pathway elucidation [13] and stimulation of resistance mechanism in plants.[14] Biologically active long chain fatty acids exert their effect on membrane phospholipids by modifying its composition and potentially replacing or interfering with the synthesis of phospholipid derived lipid signalling molecules, including endocannabinoids.[15]

The level of n-hexadecanoic acid (18.71%), 9-octadecenoic acid, methyl ester, (E)-(7.83%) and oleic acid (25.92%) in dichloromethane fraction in EG is high (Table 2). These sets of compound were also identified in the ethanol fraction. High level of hexadecanoic acid has been identified in root samples of oil palm and proven to play a defence role mechanisms.[16] Various pharmacological activities of EG constituents have been reported such as unsaturated fatty acid and linolenic acid possess antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic and antihistimic.[17]

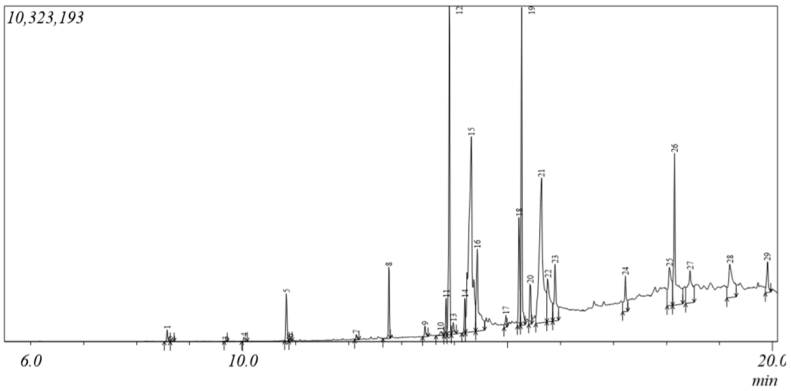

Figure 1. Peaks from the chromatogram obtained from the ethyl acetate fraction of EG fruits.

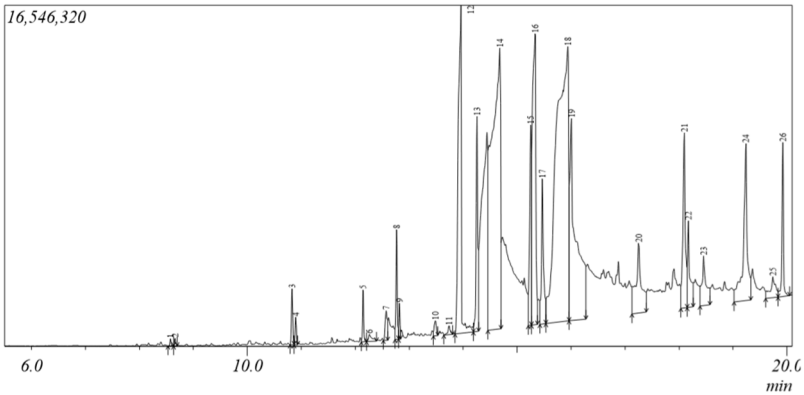

Figure 2. Peaks from the chromatogram obtained from the dichloromethane fraction of EG fruits.

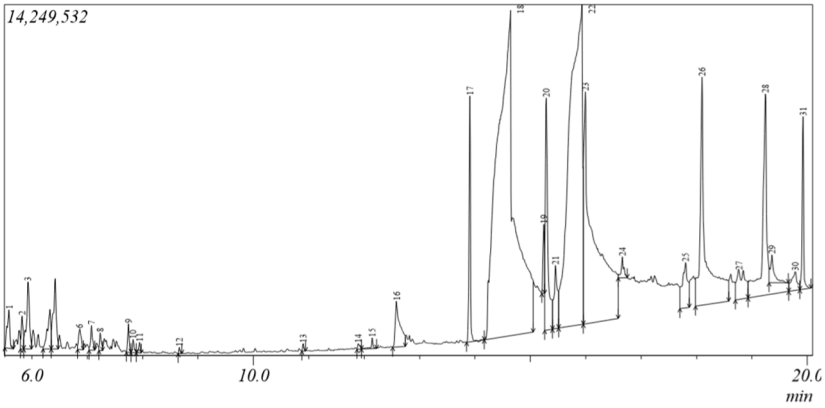

Figure 3. Peaks from the chromatogram obtained from the n-hexane fraction of EG fruits.

Table 1. Compounds that were identified from the ethyl acetate fraction of EG using GC-MS analysis.

S/N	Compounds	% Area	RT (s)	CAS	MW	Class	Identification ion	Confirmation ion
1	9-Octadecene, (E)-	0.38	8.63	7206-25-9	252	Alkene	55	69 83
2	Nonane, 3,7-dimethyl-	0.04	8.71	17302-32-8	156	Alkane	57	71 85
3	2-Bromononane	0.02	9.71	2216-35-5	206	Organo-halide	43	57 71
4	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.14	10.09	96-76-4	206	Alcohol	191	57 206
5	1-Hexadecanol	1.42	10.88	36653-82-4	242	Alcohol	55	69 83
6	Tetratetracontane	0.08	10.93	7098-22-8	618	Alkane	57	43 71
7	Cyclopentanetridecanoic acid, methyl ester	0.16	12.19	24828-61-3	296	Ester	74	55 87
8	1-Nonadecene	2.14	12.79	18435-45-5	266	Alkene	59	83 97
9	Phthalic acid, butyl undecyl ester	0.38	13.50	0-00-0	376	Ester	149	57 71
10	7-Hexadecenal, (Z)-	0.23	13.80	56797-40-1	238	Aldehyde	57	71 83
11	Phthalic acid, isobutyl undecyl ester	1.00	13.86	0-00-0	376	Ester	149	57 223
12	Hexadecanoic acid, methyl ester	10.53	13.94	112-39-0	270	Ester	74	55 87
13	Phthalic acid, butyl undecyl ester	0.48	14.03	:0-00-0	376	Ester	149	57 223
14	Cyclohexanecarboxaldehyde, 6-methyl-3-(1-methylethyl)-2-oxo-	1.03	14.21	28745-06-4	182	Aldehyde	140	55 67
15	n-Hexadecanoic acid	18.75	14.40	57-10-3	256	Alkanoic acid	73	55 60
16	n-Nonadecanol-1	4.87	14.57	1454-84-8	284	Alcohol	57	83 97
17	Phthalic acid, butyl undecyl ester	0.44	14.99	0-00-0	376	Ester	149	57 104
18	9,12-Octadecadienoic acid, methyl ester	3.59	15.24	2462-85-3	294	Ester	67	55 81
19	9-Octadecenoic acid, methyl ester, (E)-	9.46	15.33	1937-62-8	296	Ester	55	69 74
20	Heptadecanoic acid, 10-methyl-, methyl ester	1.31	15.46	2490-25-7	298	Ester	74	55 87
21	Oleic Acid	14.29	15.74	112-80-1	282	Alkanoic acid	55	69 83
22	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	3.48	15.85	117132-08-8	212	Ketone	55	57 68
23	n-Tetracosanol-1	3.31	15.97	506-51-4	354	Alcohol	55	83 97
24	17-Pentatriacontene	1.82	17.27	6971-40-0	490	Alkene	57	55 97
25	7-Hexadecenal, (Z)-	3.47	18.12	56797-40-1	238	Aldehyde	57	55 68
26	Bis-(2-ethylhexyl) phthalate	7.79	18.31	117-81-7	390	Ester	149	57 167
27	17-Pentatriacontene	3.97	18.53	6971-40-0	490	Alkane	57	55 71
28	7-Hexadecenal, (Z)-	4.03	19.32	56797-40-1	238	Aldehyde	55	57 69
29	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca	1.44	19.97	0-00-0	428	Alkane	69	57 81

Table 2. Compounds that were identified from the Dichloromethane fractions of EG using GC-MS analysis.

S/N	Compounds	% Area	RT (s)	CAS	MW	Class	Identification ion	Confirmation ion
1	9-Octadecene, (E)-	0.08	8.63	7206-25-9	252	Alkene	55	69 83
2	Hexadecane	0.08	8.70	544-76-3	226	Alkane	57	71 85
3	9-Eicosene, (E)-	0.52	10.87	74685-29-3	280	Alkene	55	83 97
4	Hexadecane	0.24	10.93	629-94-7	296	Alkane	57	71 85
5	Methyl tetradecanoate	0.52	12.21	124-10-7	242	Ester	74	55 87
6	Isolongifolan-7-ol	0.19	12.39	0-00-0	222	Alcohol	207	57 222
7	Tetradecanoic acid	0.51	12.60	544-63-8	228	Acid	73	55 60
8	1-Nonadecene	1.17	12.80	18435-45-5	266	Alkane	55	83 97
9	Nonadecane	0.33	12.84	629-92-5	268	Alkane	57	71 85
10	Oxirane, hexadecyl-	0.25	13.53	7390-81-0	268	Heterocycle	83	55 61
11	7-Hexadecenoic acid, methyl ester, (Z)-	0.15	13.81	56875-67-3	268	Ester	55	69 83
12	Hexadecanoic acid, methyl ester	7.95	14.19	112-39-0	270	Ester	74	55 87
13	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	2.99	14.28	85-69-8	334	Ester	149	55 104
14	n-Hexadecanoic acid	18.71	14.70	57-10-3	256	Acid	73	55 60
15	9,12-Octadecadienoic acid, methyl ester	2.91	15.27	2462-85-3	294	Ester	67	55 81
16	9-Octadecenoic acid, methyl ester, (E)-	7.83	15.38	1937-62-8	296	Ester	55	69 97
17	Methyl stearate	2.41	15.53	112-61-8	298	Ester	74	55 87
18	Oleic Acid	25.92	15.97	112-80-1	282	Acid	55	69 97
19	Octadecanoic acid	9.85	16.28	57-11-4	284	Acid	55	73 129
20	n-Tetracosanol-1	3.00	17.39	506-51-4	354	Alcohol	55	83 97
21	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3.48	18.15	23470-00-0	330	Ester	98	57 74
22	Bis(tridecyl) phthalate	1.65	18.27	119-06-2	530	Aromatic	149	57 167
23	17-Pentatriacontene	1.60	18.58	6971-40-0	490	Alkene	55	83 97
24	Oleoyl chloride	4.82	19.33	112-77-6	300	Organo-halide	55	69 81
25	17-Pentatriacontene	0.82	19.83	111-02-4	410	Alkene	69	81 95

Table 3. Compounds that were identified from the n-hexane fraction of EG using GC-MS analysis.

S/N	Compounds	% Area	RT (s)	CAS	MW	Class	Identification ion	Confirmation ion
1	Benzene-1,2,4,5-tetramethyl-	0.57	5.67	95-93-2	134	Aromatic	119	91 134
2	Benzene, 1-methyl-2-(2-propenyl)-	0.33	5.850	1587-04-8	132	Aromatic	117	91 132
3	2,4-Dimethylstyrene	1.13	5.992	2234-20-0	132	Alkane	117	91 132
4	Benzene, 1-methyl-4-(1-methyl-2-propenyl)-	0.72	6.350	97664-18-1	146	Aromatic	131	91 146
5	2-Naphthalenol, 1,2-dihydro-, acetate	1.18	6.475	132316-80-4	188	Aromatic	128	132 146
6	2-Ethyl-2,3-dihydro-1H-indene	0.30	6.925	56147-63-8	146	Aromatic	117	131 146
7	1H-Indene, 2,3-dihydro-4,7-dimethyl-	0.26	7.133	6682-71-9	146	Aromatic	131	115 146
8	Naphthalene, 2-methyl-	0.24	7.792	91-57-6	142	Aromatic	142	71 115
9	2,4-Decadienal, (E,E)-	0.14	7.883	25152-84-5	152	Aldehyde	81	55 67
10	Naphthalene, 1-methyl-	0.11	7.975	90-12-0	142	Aromatic	142	115 139
11	Hexadecane	0.04	8.708	544-76-3	226	Alkane	57	71 85
12	2-methyltetracosane	0.05	10.942	0-00-0	352	Alkane	57	43 71
13	Cyclopentanetridecanoic acid, methyl ester	0.12	12.225	24828-61-3	296	Ester	74	55 87
14	Tetradecanoic acid	1.23	12.742	544-63-8	228	Acid	73	55 60

15	Hexadecanoic acid, methyl ester	2.06	14.167	112-39-0	270	Ester	74	55 87
16	n-Hexadecanoic acid	31.41	15.050	57-10-3	256	Acid	60	55 73
17	9,12-Octadecadienoic acid (Z,Z)-	0.57	15.258	60-33-3	280	Acid	67	55 81
18	9-Octadecenoic acid (Z)-, methyl ester	3.19	15.400	112-62-9	296	Ester	55	69 83
19	Methyl 9-methyltetradecanoate	1.22	15.508	213617-69-7	256	Ester	74	55 87
20	cis-Vaccenic acid	22.82	15.958	506-17-2	282	Acid	55	69 83
21	Octadecanoic acid	13.16	16.583	57-11-4	284	Acid	55	57 73
22	Cyclopentadecanone, 2-hydroxy	0.28	16.750	4727-18-8	240	Ketone	55	69 83
23	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	7.00	18.583	23470-00-0	330	Ester	98	57 74
24	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	1.35	18.933	4575-74-0	270	Ester	69	55 159
25	Oleoyl chloride	6.17	19.667	112-77-6	300	Organo-halide	55	67 81
26	Methyl 12-oxo-9-dodecenoate	0.59	19.658	22418-58-2	226	Ester	55	69 98
27	7-Hexadecenal, (Z)-	0.58	19.87	56797-40-1	238	Aldehyde	55	69 83
28	Squalene	1.59	20.07	111-02-4	410	Alkene	69	81 95

In the *n*-hexane fraction (Table 3), n-hexadecanoic acid (31.41%), cis-vaccenic acid (22.82%) or 11-octadecenoic acid and octadecanoic acid (13.16%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (7.00%) and oleoyl chloride (6.17%) were identified. Vaccenic acid potentiate complications seen in dyslipidemia, fatty liver disease, and low-grade inflammation.[18] It has been proposed that the lipid-lowering and anti-inflammatory effects of vaccenic acid may be partially associated with its ability to ligand activate PPARγ-regulated pathways, by acting directly in the intestine and adipose tissue.[14] Furthermore, given the bioactive properties of vaccenic acid to favourably modulate whole body energy metabolism and low-grade inflammation.[14]

Esters with methyl attached to the fatty acid are mostly grouped as fatty acid methyl ester and has shown susceptibility to isolates of Paracoccidioides (MIC= 15.6 µg/ml). Antimicrobial activity have been reported for oil with stearic, palmitic, oleic, linoleic and linolenic acids components,[19] this could be attributed to the presence of fatty acids.[12]

CONCLUSION

The use of GC-MS analysis in profiling EG fruit for its chemical content is highly revealing, because it gave a quick opportunity for the various molecular weights of different compounds present in the fractions of EG extract to be identified. From the parent molecular ions, the approximate molecular structure of the different compounds identified was obtained. Profiling of EG have provided an idea of its chemical composition which include long and short chain fatty acids, alkane, alkene, alcohols, aldehyde and esters. These compounds are known to possess different pharmacological activity both in the plant and animals.

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Contribution to the Study. This work is an undergraduate project work for Pharm (Dr.) Mike Oshor Ighene. It was supervised Dr Emmanuel E. Odion and Co-supervised by Rachel O. Ogboru. The idea of the work was conceived by Emmanuel Odion and Rachel Ogboru, Mike conducted the experiment under the guidance of Emmanuel Odion, data were analyzed by Emmanuel and Rachel. Write up was done by Emmanuel and Rachel.

REFERENCES

1. Hartley, C.W.S., 2000. *The Oil Palm*. 3rd Edition, Longman Press London and New York, p. 806.
2. Naher, L., Yusuf, U.K., Ismail, A., Tan, S.G. and Mondal, M.M.A. 2013. Ecological status of Ganoderma and basal stem root disease of oil palms (*Elaeis guineensis* Jacq.). *Australian J. Crops Sci.* **7**, 1723-1727.
3. Corley, R.H.V. 1976. The Genus *Elaeis*. In: *Oil Palm Research* (Corley, R.H.V., Hardon, J. J. and Wood, B. J. Eds.), Elsevier Scientific Publisher Amsterdam, Netherlands. p. 532.
4. Ekwenye, U.N. and Ijeomah, C.A. 2005. Antimicrobial effects of palm kernel oil and palm oil. *King Mongkut's Inst. Tech. Ladk. Sci. J.* **5**, 502-505.
5. vin, T.T. 1985. Wound healing. *Arch. Emerg. Med.* **2**, 3-10.
6. Sasidharan, S., Sharmini, R., Vijayarathna, S., Yoga Latha, L., Vijenthi R, Amala R. and Amutha S. 2009. Antioxidant and hepatoprotective activity of methanolic extracts of *Elaeis guineensis* Jacq leaf. *Pharmacol. Online.* **3**, 84-90.
7. Alder, L., Greulich, K., Kempe, G. and Vieth, B. 2006. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS?. *Mass Spectrum Rev.* **25**, 838-865.
8. Hernando, M.D., Aguera, A., Fernandez-Alba, A.R., Piedraa L. and Contrerasb, M. 2001. Gas chromatographic determination of pesticides in vegetable samples by sequential positive and negative chemical ionization and tandem mass spectrometric fragmentation using an ion trap analyser. *Anal.* **126**, 46-51.
9. Chauhan, A., Goyal, M.K. and Chauhan, P. 2014. GC-MS technique and its analytical applications in science and technology. *J. Anal. Bioanal. Tech.* **5**, 222.
10. Kamatou, G.P.P. and Viljoen A.M. 2017. Comparison of fatty acid methyl esters of palm and palmist oils determined by GCxGC–ToF–MS and GC–MS/FID. *South Afr. J. Bot.* **112**, 483-488.
11. Rozali, N.L., Yarmo, M.A., Idris A.S., Kushairi, A. and Ramli, U.S. 2017. Metabolomics differentiation of oil palm (Elaeis guineensis Jacq.) spear leaf with contrasting susceptibility to *Ganoderma boninense*. *Plant Omics J.* **10**, 45-52.
12. Desbois, A.P. and Smith, V.J. 2009. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl. Microbiol. Biotech.* **85**, 1629-1642.
13. Farmer, E.E., Weber, H. and Vollenweider, S. 1998. Fatty acid signalling in *Arabidopsis*. *Planta.* **206**, 167-174.
14. Jacome-Sosa, M. M., Lu, J., Wang, Y., Ruth, M.R., Wright, D.C., Reaney, M. J., Shen, J., Field, C.J., Vine, D. F. and Proctor, S. D. 2010. Increased hypolipidemic benefits of cis-9, trans-11 conjugated linoleic acid in combination with trans-11 vaccenic acid in a rodent model of the metabolic syndrome, the JCR:LA-cp rat. *Nutr. Metab.***7**, 60.
15. Fernandis, A. Z. and Wenk, M. R. 2007. Membrane lipids as signaling molecules. *Curr. Opin. Lip.* **18**, 121-128.
16. Nusaibah, S.A., Akmar, S.N. A., Idris, A.S., Sariah, M. and Mohamad, P. Z. 2016. Involvement of metabolites in early defence mechanism of oil palm *(Elaeis guineensis Jacq.)* against Ganoderma disease. *Plant Physiol. Bioch.* **109**, 156-165.
17. Sahreen, S, Khan, M.R. and Khan, R.A. 2010. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chem.***122**, 1205-1211.
18. Wang, Y., Lu, J., Ruth, M.R., Goruk, S.D., Reaney, M.J., Glimm, D.R., Vine, D.F., Field, C.J. and Proctor, S.D. 2008. Trans-11 vaccenic acid dietary supplementation induces hypolipidemic effects in JCR:LA-cp rats. *J. Nutr.* **138**, 2117-2122.
19. Ogawa, J., Kishino, S., Ando, A., and Satoshi Sugimoto, S. 2005. Review: production of conjugated fatty acids by lactic acid bacteria. *J. Biosci. Bioeng.* **100**, 355-364.