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BIOACTIVE COMPOUNDS DETERMINATION USING GC-MS ANALYSIS OF PANDANUS AMARYLLIFOLIUS ROXB. LEAVES

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ABSTRACT

Pandanus Roxb. a medicinally important plant belongs to the Family Pandanaceae. It is an important leafy vegetable and the leaves are widely used in Indian cookery for flavouring foodstuffs. Green leafy vegetables are a blessing for a safe and healthier life and have been in use for centuries. It provides vital nutrients require for human health and wellbeing. Since ancient times, green leafy vegetables have been used as medicine and have a great impact of our diet and nutrition. Gas chromatography-mass spectroscopy (GC-MS) is a tool capable of identifying active principles in plant's extractions. Plants are the natural source of bioactive components responsible for the medicinal efficacy due to the presence of by product. 81 compounds were detected in ethanol extract of *Pandanus amaryllifolius*. Squalene was identified as the major compound.

Keywords: *Pandanus amaryllifolius,* GC-MS, Squalene

INTRODUCTION

Green leafy vegetables are a blessing for safe and healthier life and have been in use for centuries (Randhawa, 2015). Herbs are plant valued for their medicinal and aromatic properties and often grown and harvested for these unique properties. In most parts of the world, herbs are grown mainly as field crops or on small scale as catch crop among vegetables. The knowledge on herbs has been handed down from generation to generation thousands of years (Brown, 1995).

The genus *Pandanus* from the family Pandanaceae comprises approximately 600 species that are widely distributed in tropical and subtropical regions (Takayama *et al*., 2002). The leaf of *Pandanus amaryllifolius Roxb*., commonly known as pandan, is often used to give a refreshing, fragrant flavor (Ampa Jimtaisong and Panvipa Krisdaphong, 2012). It is an evergreen

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perennial aromatic plant, a cultivated plant now found worldwide due to importation and human migration (Keller, 2001).

Gas chromatography-mass spectroscopy (GC-MS) is a tool capable of identifying active principles in plant's extractions. It identifies compounds such as fatty acids, steroids, aromatic compounds, and non-polar components with high separation efficiency and sensitivity to detect components in a mixture especially on volatiles compounds(Li *et al*., 2013;Gomathi *et al*., 2015). Volatile substances are the main factors responsible for aroma, which belong with other factors such as taste and physical factors contribute to the flavor (Baroni *et al*., 2006)

MATERIALS AND METHODS

Systematic position

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliospida
Order	:	Pandanales
Family	:	Pandanaceae
Genus	:	*Pandanus*
Species	:	*amaryllifolius*

Sample collection and solvent extraction

The *Pandanus amaryllifolius* plant was selected for the GC-MS study. It was cultivated in the kitchen garden. The plant was dried under shade condition for one month and pulverized into fine powder and filtered through a mesh. The extraction was made using ethanol.

Gas chromatography-mass spectrometry (gc-ms) analysis

GC -MS (QP-ultra-2010, Shimadzu, Japan) analysis was carried out for fatty acid methyl esters using SH-Rxi- 5Sil MS (30m,0.25mm, 0.25 µm Columns (low- polarity phase; Crossbond

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1,4 -bis (Dimethylsiloxy) phenylene dimethyl polysiloxane) with electron impact (EI) ionization. Helium was used as a carrier gas at 1.5ml min-1. In GC, injection temperature was maintained at 280ºC.

The oven temperature profile was at initial temperature with 70ºC hold 1 min, increase 5ºC/min up to 255ºC and hold 3min, further increase 5ºC/min up to 300ºC holding time 5 minutes. The total programme time was 54 minutes. The split ratio was 1:10 and the column flow parameter was 1ml/min. In MS, ion source temperature was 230ºC and interface temperature was 280ºC in scan mode with m/z detection from 35-850 Da.

RESULT & DISCUSSION

Table:1 Gas Chromatography-Mass spectrometry (GC-MS) analysis of *Pandanus amaryllifolius* using ethanol extract

Peak#	R.Time	Area%	Height%	A/H	MW	Name
1	5.411	0.34	0.29	7.27	102	Methyl butanoate
2	6.300	0.33	0.26	7.85	114	2,5-Furandione, dihydro-3-methyl-
3	6.778	0.07	0.08	5.24	144	Butanoic acid, 2-methyl-3-oxo-, ethyl ester
4	8.069	0.26	0.38	4.34	142	Decane
5	8.678	0.11	0.14	5.00	244	Decanoic acid-TMS
6	9.274	0.85	0.64	8.28	202	Mevalonic lactone-TMS
7	11.342	0.38	0.31	7.56	120	Benzofuran, 2,3-dihydro-
8	12.279	0.58	0.55	6.62	232	1-Propoxy-2-propanol, TBDMS derivative
9	13.190	0.15	0.14	6.74	188	Caproic acid-TMS
10	13.663	0.08	0.09	5.43	282	Methyl cis-10-heptadecenoate
11	13.892	0.60	0.47	7.86	276	Methylsuccinic acid, 2TMS derivative
12	15.452	0.18	0.13	8.41	276	Ureidopropionic acid-2TMS
13	15.778	0.21	0.26	5.21	276	Ureidopropionic acid-2TMS
14	17.608	0.05	0.08	4.18	276	2-Hydroxyisocaproic acid-2TMS
15	18.135	0.37	0.28	8.25	102	Methyl butanoate
16	18.355	0.31	0.18	10.60	244	Decanoic acid-TMS
17	18.776	0.91	0.76	7.40	202	Mevalonic lactone-TMS

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18	19.467	0.20	0.17	7.38	203	Acetoacetic acid-meto-TMS
19	19.806	0.30	0.33	5.63	102	Methyl butanoate
20	20.193	0.14	0.15	5.83	376	Arachidonic acid-TMS
21	20.323	0.05	0.07	4.28	254	Methyl cis-10-pentadecenoate
22	20.983	9.24	4.43	12.97	156	5-Hydroxycyclooctane-1,2-dione
23	21.841	0.05	0.09	3.45	272	Lauric acid-TMS
24	22.247	0.14	0.14	5.99	102	Methyl butanoate
25	23.249	0.77	0.32	14.98	102	Methyl butanoate
26	24.227	0.20	0.18	7.15	102	Methyl butanoate
27	24.423	0.13	0.15	5.41	294	Methyl linoleate
28	25.563	0.11	0.15	4.88	220	Cinnamic acid-TMS
29	25.703	0.11	0.13	5.24	354	Oleic acid-TMS
30	25.832	1.16	1.43	5.04	278	Neophytadiene
31	25.959	0.23	0.26	5.43	296	Methyl oleate
32	26.321	0.33	0.33	6.32	198	11-Tridecen-1-ol
33	26.705	0.31	0.39	4.93	278	Neophytadiene
34	28.294	4.51	4.69	5.99	242	Pentadecanoic acid
35	29.841	0.41	0.31	8.43	300	Myristic acid-TMS
36	30.190	0.10	0.13	4.83	354	Oleic acid-TMS
37	31.084	0.81	0.99	5.06	296	Phytol
38	31.343	0.02	0.04	3.81	488	Batyl alcohol-2TMS
39	31.473	1.31	1.97	4.12	322	11,14-Eicosadienoic acid, methyl ester
40	31.574	7.50	5.71	8.16	278	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
41	32.038	0.69	0.48	8.87	354	Oleic acid-TMS
42	32.207	0.25	0.25	6.18	352	Linoleic acid-TMS
43	32.970	0.89	0.55	9.97	376	Arachidonic acid-TMS
44	34.479	0.06	0.09	3.95	175	Dimethylglycine-TMS
45	35.601	0.15	0.15	6.21	320	Methyl cis-11,14,17-Icosatrienoate
46	38.437	0.09	0.12	4.86	175	Dimethylglycine-TMS
47	39.339	0.07	0.10	4.61	102	Methyl butanoate
48	39.476	0.11	0.12	5.94	102	Methyl butanoate
49	39.933	0.25	0.26	6.01	226	Pentadecanal
50	42.420	0.19	0.21	5.63	374	Eicosapentaenoic acid-TMS

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51	42.753	0.67	0.46	9.21	254	Methyl cis-10-pentadecenoate
52	42.946	0.27	0.32	5.25	282	Nonadecane, 9-methyl-
53	43.505	0.18	0.17	6.59	354	Oleic acid-TMS
54	44.020	0.01	0.02	2.62	262	3-Hydroxyisovaleric acid-2TMS
55	44.561	0.14	0.19	4.45	352	2-Methyltetracosane
56	44.714	23.79	31.34	4.72	410	Squalene
57	45.063	0.13	0.12	6.44	376	Arachidonic acid-TMS
58	46.062	1.23	1.44	5.32	380	2-Methylhexacosane
59	46.287	1.66	2.17	4.74	426	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,
60	46.439	0.66	0.82	5.06	446	2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetrac
61	47.085	0.06	0.08	4.97	322	Methyl cis-11,14-Icosadienoate
62	47.245	0.37	0.46	4.90	290	trans-Geranylgeraniol
63	47.485	1.94	1.61	7.47	352	2-Methyltetracosane
64	48.080	1.28	1.52	5.27	416	gamma.-Tocopherol
65	48.636	0.13	0.14	5.92	376	Arachidonic acid-TMS
66	48.841	4.19	5.35	4.87	618	Tetratetracontane
67	49.130	6.37	5.50	7.20	430	dl-.alpha.-Tocopherol
68	50.129	2.50	2.23	6.99	380	2-Methylhexacosane
69	50.409	2.35	1.20	12.20	458	Cholesterol-TMS
70	50.777	4.84	3.55	8.48	412	Stigmasterol
71	51.024	0.90	0.69	8.09	400	Docosahexaenoic acid-TMS
72	51.522	4.25	5.23	5.05	618	Tetratetracontane
73	51.678	3.51	3.66	5.96	414	.beta.-Sitosterol
74	51.902	0.83	0.67	7.66	352	Linoleic acid-TMS
75	52.077	0.03	0.06	3.48	240	Methyl myristoleate
76	53.119	0.42	0.43	6.03	488	Batyl alcohol-2TMS
77	53.290	0.06	0.09	3.92	376	Arachidonic acid-TMS
78	53.463	0.13	0.15	5.43	262	3-Hydroxyisovaleric acid-2TMS
79	53.566	0.24	0.24	6.21	488	Batyl alcohol-2TMS
80	53.779	0.08	0.10	5.25	248	4-Hydroxybutyric acid-2TMS
81	54.067	0.10	0.07	8.91	376	Arachidonic acid-TMS

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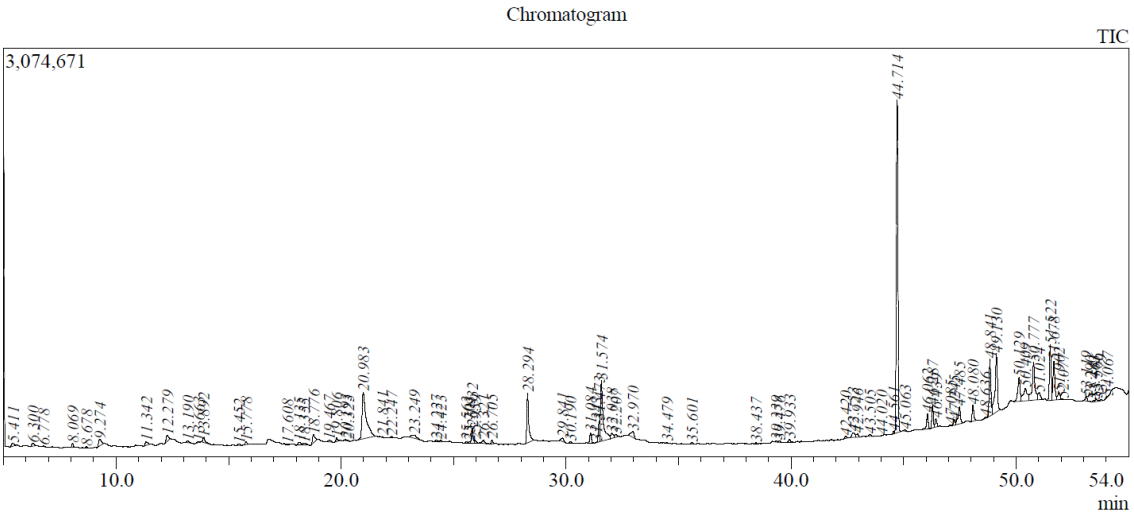
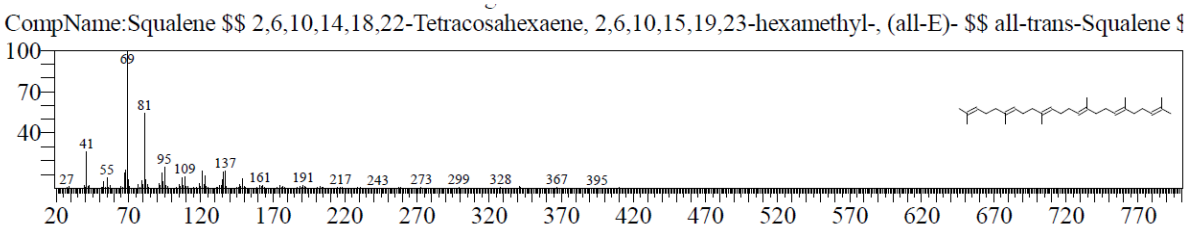
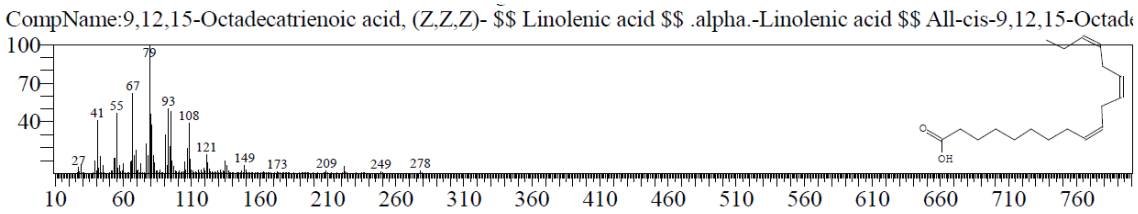
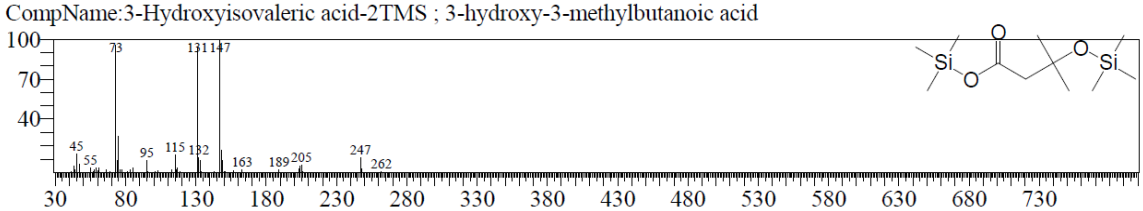
Figure: 1 Gas Chromatography-Mass spectrometry (GC-MS) analysis of *Pandanus amaryllifolius* using ethanol extract



*Min - Minutes *TIC – Total Ion Chromatogram







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Bioactive compounds determination by Gas Chromatography – Mass spectrometry (GC-MS) analysis of *Pandanus amaryllifolius* using ethanol extract

The result on GC-MS analysis in ethanol extract of *Pandanus amaryllifolius* with their retention time was showed in Table 1and Figure 1. 81 compounds were detected in ethanol extract of *Pandanus amaryllifolius*. In retention time 44.714 min, the extraction compound squalene occurred with highest peak area of 23.79% which the molecular weight 410. The compound 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- showed the medium peak area of 7.50% at retention time 31.574 min with 278 molecular weight. In retention time 44.020 min, the extraction compound 3-Hydroxyisovaleric acid-2 TMS was observed with lowest peak area (0.01%) which has molecular weight 262. Maisarah Mohamed Zakaria *et al*., 2020 discussed the squalene was identified as the major compound of Eos which contributed to the highest percentage area 14.14%, 33.83% and 16.92% of Eos from kedah, Selanor, and Johor, respectively.

CONCLUSION

Pandanus leaves have the strongest flavor and aroma. From the result, It can be concluded that the plant extract show the presence of 81 phyto compounds. The presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners.

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