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Original Article

GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY) ANALYSIS SEED KEBIUL (*CAESALPINIA BONDUC* (L) ROXB)

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ABSTRACT

Objective: Kebiul (*Caesalpinia bonduc* (L) Roxb) is one of the medicinal plants that plays an important role in treating various diseases, but this plant is vulnerable to extinction. This study aims to explore the possibility of various biological compounds in the ethanol extract of the seed kernel and seed coat of kebiul using gas chromatography-mass spectrometry (GC-MS) analysis.

Methods: The seed coat was removed from the kernel of the kebiul seed. After being ground into a powder, the seed kernel and seed coat were dried in an oven set to 40 °C for 1 h, and grinding them into a fine powder. The powder was soaked in 70% ethanol at a 1:10 (w/v) ratio for 3 days at room temperature, with occasional stirring. The chemical composition of secondary metabolite in the ethanol extracts of both the seed kernel and seed coat of kebiul was analyzed using gas chromatography–mass spectrometry (GC-MS) instrumentation (ID ISQD1702517_1) at a temperature of 25.30 °C and humidity of 35%.

Results: In this GC-MS analysis, 87 bioactive phytochemical compounds were identified in the ethanol extract of seed kernel and 50 bioactive phytochemical compounds were identified in the ethanol extract of seed coat. Seven chemicals in the seed kernel ethanol extract obtained the highest relative peak area (>2%) from each chromatogram, with the highest compound at a retention time of 19.93 min, namely 6-Octadecenoic acid, and three compounds in the seed coat ethanol extract obtained the highest relative peak area (>2%) in this investigation, with the highest compound at a retention time of 19.65 min, namely 9,12-Octadecadienoic acid (Z,Z)-.

Conclusion: GC-MS analysis identified 87 phytochemical compounds were identified in the ethanol extract of kebiul seed kernel and 50 phytochemical compounds were identified in the ethanol extract of kebiul seed coat.

Keywords: Active compound, *Caesalpinia bonduc* (L) Roxb, GC-MS analysis

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INTRODUCTION

In traditional medicine, various parts of the plant are consumed alone and formulated as polyherbs, along with other medicinal plants, which are further used to treat various diseases [1]. Kebiul seeds are bulging oblong pods measuring 5 to 7.5 cm, covered with spines, and containing one or two seeds per pod. The pods have short stems, and the seeds are 1-2 cm in size, round, hard, and bluish-gray in color with a smooth shiny surface [2]. All parts of the plant (roots, leaves, seeds, bark, and stem) have been used as herbal medicine for various diseases such as antipyretic, antiperiodic, anthelmintic, antibacterial, hydrocele, antioxidant, antitumor, and antidiabetic and also used for the treatment of skin diseases such as leprosy, as well as used for the treatment of paralysis and nervous complaints [2-4].

Medicinal plants are more prone to extinction [5]. However, the ability to regenerate entire plants from epicotyl explants using callus induction and shoot regeneration methods has been demonstrated. This provides an efficient and reproducible protocol for the regeneration of ethnomedicinal plants, which is important for *in vitro* propagation and large-scale species conservation [6]. Therefore, identifying the active ingredients in traditional medicinal plants used as therapeutic drugs is necessary for scientific validation purposes [7]. Gas Chromatography-Mass Spectrometry (GC-MS) has become a popular technique to identify volatile and non-volatile compounds in plant species. Many previous studies found phytochemical compounds in plant components using the GC-MS method [8]. There is currently relatively little scientific data on the characterization of chemicals found in seed kernels and seed coat kebiul. This study is an early attempt to use the GC-MS method to determine the secondary metabolite profile, which can reveal additional details about the distribution of bioactive chemicals in both seed sections. The main objective of this study is to identify the biological compounds present in the seed kernel and seed coat of kebiul, which are extracted using 70% ethanol and analyzed with the GC-MS method, allowing for their potential to be assessed through *in vitro* and *in vivo* tests aimed at discovering new compounds that may have therapeutic applications. Using different methods and solvents is expected to produce different compounds and concentrations.

MATERIALS AND METHODS

Sample preparation

Kebiul seeds from woods on Sumatra Island, Indonesia's Bengkulu province, served as the samples. At the General Hospital's Tawangmangu Traditional Health Service Functional Service Unit, tests for plant determination were conducted. Test report number TL.02.04/D. XI.6/22566.1061/2024, with synonym *Caesalpinia bonduc* (L) Roxb, was submitted by Dr. Sarjito of Karanganyar Regency, Central Java. After being separated into seed kernels and seed coats, the sample was ground into a powder. The seed coat was removed from the kernel of the kebiul seed. After being ground into a powder, the seed kernel and seed coat were dried in an oven set to 40 °C for 1 h, and grinding them into a fine powder. The powder was soaked in 70% ethanol at a 1:10 (w/v) ratio for 3 days at room temperature, with occasional stirring. After maceration, the mixture was filtered using whatman No. 1 filter paper. The residue was re-macerated twice under the same conditions. All filtrates were pooled and evaporated under reduced pressure at 40 °C using a rotary

evaporator. The resulting extract was then stored in a sealed container at 4 °C for subsequent analysis.

Instrument GC-MS

This research is an exploratory study using ethanol extract from the seed kernel and seed coat of kebiul. The chemical composition of secondary metabolite in the ethanol extracts of both the seed kernel and seed coat of kebiul was analyzed using gas chromatography–mass spectrometry (GC-MS) instrumentation (ID ISQD1702517_1) at a temperature of 25.30 °C and humidity of 35%. The analysis was performed using a capillary column HP-5MS (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The injection volume was 1 µl** with a split ratio of 10:1. The GC oven temperature was programmed as follows: initial temperature 50 °C (held for 2 min), ramped to 280 °C at 10 °C/min, and held at 280 °C for 10 min. GC-MS analysis was conducted using electron ionization at 70 eV, scanning m/z values from 40 to 600. Detector and injector were maintained at 280 and 250 °C, respectively. Then, the compound identification was performed by comparing retention indices and mass spectra with entries in the National Institute of Standards and Technology (NIST) library database, as described [9].

RESULTS

By employing the maceration method to produce 70% ethanol extract of the seed kernel and seed coat of kebiul, 29.99 g of ethanol extract of the seed kernel with a yield of 2.14% and 26.81 g of ethanol extract of the seed coat of kebiul with a yield of 2.6% were obtained. The chromatogram of the kebiul seed kernel, along with its corresponding retention periods and peaks, was obtained by GC-MS analysis and is shown in fig. 2 and table 1.

Fig. 1: Preparation of seed kernel and seed coat of kebiul. a. seeds, b. seed coat, c. seed kernel of kebiul

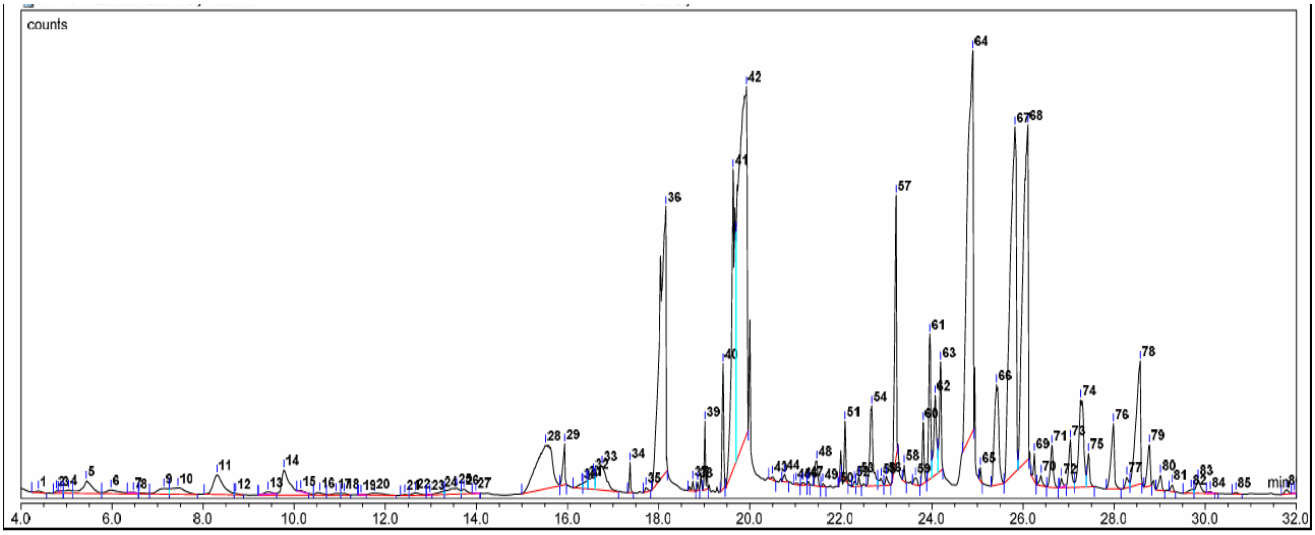


Fig. 2: Chromatogram of ethanol extract of kebiul seed kernel by GC-MS

Fig. 2 shows the chromatogram identified in the ethanol extract of the seed kernel. Individual compounds were identified by comparing their mass spectra database with the main library alliance, ensuring the listed spectra had a Similarity Index (SI) of more than 80% (NIST), and by comparing them with values published in the literature. The GC-MS analysis indicated that 87 phytochemical compounds were identified in the ethanol extract of seed kernels (tables 1 and 2).

Table 1: Compound of ethanol extract of kebiul seed kernel by GC-MS

No.	Ret. time min	Hit# 1	Chemical formula	Mol. weight	SI Hit#1	Rel. area %	Area counts*min
1	4,38	Sec-Butyl nitrite	$C_4H_9NO_2$	103	652	0,05	12356481,038
2	4,78	2-Nitro-1-buten-3-ol	$C_4H_7NO_3$	117	686	0,09	21175346,480
3	4,87	Isoxazolidine-3,5-dicarboxylic acid, dimethyl ester	$C_7H_{11}NO_5$	189	678	0,01	1435204,059

No.	Ret. time min	Hit# 1	Chemical formula	Mol. weight	SI Hit#1	Rel. area %	Area counts*min
4	5,05	N-Isopentyl-N-nitroso-pentylamine	$C_{10}H_{22}N_2O$	186	664	0,13	30663445,304
5	5,43	Propanoic acid, 3-ethoxy-, ethyl ester	$C_7H_{14}O_3$	146	736	0,69	166938220,038
6	5,98	Alpha-l-rhamnopyranose	$C_6H_{12}O_5$	164	730	0,32	77240815,627
7	6,47	d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	210	663	0,02	4290477,110
8	6,59	2-Deoxy-D-galactose	$C_6H_{12}O_5$	164	694	0,04	8648154,764
9	7,14	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236	706	0,33	80890210,831
10	7,45	Glycerin	$C_3H_8O_3$	92	739	0,49	118153069,053
11	8,30	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	842	1,05	254430558,682
12	8,71	a-D-Galactopyranose, 2-(acetylamino)-2-deoxy-	$C_8H_{15}NO_6$	221	694	0,03	7774744,820
13	9,42	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236	747	0,09	22094672,577
14	9,77	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	853	1,29	314249191,294
15	10,15	6-Acetyl-ß-d-mannose	C8H14O7	222	710	0,03	8442761,233
16	10,56	Ascaridole epoxide	$C_{10}H_{16}O_3$	184	733	0,09	21494313,997
17	10,92	l-Gala-l-ido-octonic lactone	$C_8H_{14}O_8$	238	712	0,07	16405387,642
18	11,10	Dithiocarbamate, S-methyl-,N-(2-methyl-3-oxobutyl)-	$C_7H_{13}NOS_2$	191	732	0,05	11323892,739
19	11,47	Melezitose	$C_{18}H_{32}O_{16}$	504	787	0,00	846197,660
20	11,77	Melezitose	$C_{18}H_{32}O_{16}$	504	787	0,15	36047631,307
21	12,43	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	746	0,02	5128618,693
22	12,66	Melezitose	$C_{18}H_{32}O_{16}$	504	753	0,09	22299600,463
23	12,98	Melezitose	$C_{18}H_{32}O_{16}$	504	740	0,00	1121755,970
24	13,25	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	745	0,11	26612156,948
25	13,56	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216	740	0,38	91361996,381
26	13,72	Melezitose	$C_{18}H_{32}O_{16}$	504	741	0,14	33739231,949
27	13,99	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	775	0,01	2514248,128
28	15,52	Ethyl a-d-glucopyranoside	$C_8H_{16}O_6$	208	850	3,79	919183671,051
29	15,94	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	874	0,42	102582161,109
30	16,32	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	761	0,10	23927031,993
31	16,42	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	771	0,16	39138063,552
32	16,57	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	766	0,41	100239213,835
33	16,76	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	677	1,14	276170844,873
34	17,37	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	900	0,17	41806131,566
35	17,72	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	841	0,06	14857838,310
36	18,16	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	856	8,97	2177601507,849
37	18,75	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	850	0,10	25223422,168
38	18,86	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	817	0,05	13348651,915
39	19,02	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	923	0,47	113340275,316
40	19,41	Androsta-4,16-dien-3-one	$C_{19}H_{26}O$	270	714	0,99	240011900,417
41	19,64	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	886	5,86	1422586259,826
42	19,93	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	877	16,28	3951600262,929
43	20,50	1-Heptatriacotanol	$C_{37}H_{76}O$	536	817	0,04	9685050,657
44	20,77	1-Heptatriacotanol	$C_{37}H_{76}O$	536	825	0,09	22874119,034
45	21,02	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	$C_{25}H_{42}O_2$	374	813	0,03	6862248,149
46	21,16	1-Heptatriacotanol	$C_{37}H_{76}O$	536	811	0,05	11870633,118
47	21,29	1-Heptatriacotanol	$C_{37}H_{76}O$	536	784	0,03	6601142,798
48	21,47	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	$C_{20}H_{34}O_2$	306	780	0,37	89915392,029
49	21,60	1-Heptatriacotanol	$C_{37}H_{76}O$	536	815	0,03	6871881,498
50	21,94	1-Heptatriacotanol	$C_{37}H_{76}O$	536	811	0,03	7184095,749
51	22,09	2-Phenyl-4-tret-butyl-7-methylindene	$C_{20}H_{22}$	262	746	0,36	88592175,622
52	22,30	1-Heptatriacotanol	$C_{37}H_{76}O$	536	830	0,06	13499608,111
53	22,40	Curan, 16,17,19,20-tetradehydro-	$C_{19}H_{22}N_2$	278	664	0,09	21530222,532
54	22,68	2,4,6-Tri-t-butylbenzenethiol	$C_{18}H_{30}S$	278	679	1,35	327352977,751
55	22,89	1-Heptatriacotanol	$C_{37}H_{76}O$	536	756	0,14	33836126,080
56	23,00	1-Heptatriacotanol	$C_{37}H_{76}O$	536	807	0,11	27317859,989
57	23,21	Pregna-5,17(20)-dien-3-ol, (3ß,17E)-	$C_{21}H_{32}O$	300	728	2,52	611086882,463
58	23,39	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	765	0,10	23951481,918
59	23,64	1-Heptatriacotanol	$C_{37}H_{76}O$	536	794	0,13	31028509,408
60	23,81	Gibbane-1,10-dicarboxylic acid, 4a,7-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1a,4aa,4bß,10ß)-	$C_{20}H_{26}O_5$	346	678	0,58	139667652,116
61	23,95	1,4,9(11)-Pregnatriene-3,20-dione, 21-acetoxy-17-hydroxy-	$C_{23}H_{28}O_5$	384	668	1,51	367737480,175
62	24,07	1-Heptatriacotanol	$C_{37}H_{76}O$	536	701	1,14	275818489,090
63	24,19	17Alpha-ethynyl-17beta-hydroxy-6beta-methoxy-3alpha,5-cyclo-5alpha-androstan-19-oic acid	$C_{22}H_{30}O_4$	358	701	1,03	251033153,944
64	24,90	Pregnenolone	$C_{21}H_{32}O_2$	316	720	11,77	2858353832,214
65	25,06	6ß-Hydroxymethandienone	$C_{20}H_{28}O_3$	316	707	0,07	18100793,977
66	25,42	Norethindrone Acetate	$C_{22}H_{28}O_3$	340	730	2,36	572240692,503
67	25,82	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{28}H_{38}O_9$	518	662	11,21	2720558837,592
68	26,11	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-	$C_{28}H_{38}O_9$	518	722	9,58	2324559456,196

No.	Ret. time min	Hit# 1	Chemical formula	Mol. weight	SI Hit#1	Rel. area %	Area counts*min
		[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-					
69	26,25	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{28}H_{38}O_9$	518	681	0,21	50305606,222
70	26,40	5,16,20-Pregnatriene-3beta,20-diol diacetate	$C_{25}H_{34}O_4$	398	756	0,16	37891353,402
71	26,63	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{26}H_{36}O_8$	476	758	0,65	157287190,038
72	26,84	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{28}H_{38}O_9$	518	711	0,10	25002936,711
73	27,04	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{28}H_{38}O_9$	518	746	0,74	180109102,256
74	27,27	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{26}H_{36}O_8$	476	784	2,32	563669675,482
75	27,44	4,13,20-Tri-O-methylphorbol 12-acetate	$C_{25}H_{36}O_7$	448	762	0,42	101842917,656
76	27,99	Phorbol 12,13,20-triacetate	$C_{26}H_{34}O_9$	490	747	1,15	278563075,943
77	28,28	Acetic acid, 17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-11-yl (ester)	$C_{25}H_{34}O_5$	414	721	0,18	42548366,987
78	28,57	2-Cyclopenten-1-one, 3,4-dihydroxy-5-(3-methyl-2-butenyl)-2-(3-methyl-1-oxobutyl)-4-(4-methyl-1-oxo-3-pentenyl)-	$C_{21}H_{30}O_5$	362	650	3,00	729450089,319
79	28,77	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR-(1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]-	$C_{32}H_{39}NO_{10}$	597	698	0,61	149225718,612
80	29,02	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	$C_{24}H_{34}O_7$	434	696	0,23	56766218,653
81	29,27	Acetic acid, 17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-11-yl (ester)	$C_{25}H_{34}O_5$	414	796	0,08	19652808,230
82	29,69	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	770	0,11	26385262,922
83	29,84	Pregn-5-ene-3,11,12,14,20-pentol, 11-acetate 12-(3-methylbutanoate), (3ß,11a,12ß,14ß)-	$C_{28}H_{44}O_7$	492	717	0,38	91647832,814
84	30,11	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	$C_{24}H_{34}O_6$	418	782	0,02	6034424,424
85	30,67	Stigmasterol	$C_{29}H_{48}O$	412	766	0,03	8062119,845
86	31,78	?-Sitosterol	$C_{29}H_{50}O$	414	802	0,10	23362737,090
87	31,96	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	$C_{28}H_{40}O_4$	440	717	0,01	1880362,172

Table 2 summarizes the seven compounds in the seed kernel ethanol extract with the highest relative peak areas in this investigation, whereas fig. 4-10 summarize the apex pecan compound and real-time.

Ret. time	19,635
	Hit# 1
Component:	9,12-Octadecadienoic acid, ethyl ester
SI:	886
RSI:	928
Library:	mainlib
	Hit# 2
Component:	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
SI:	863
RSI:	886
Library:	mainlib
	Hit# 3
Component:	Butyl 9,12-octadecadienoate
SI:	862
RSI:	882

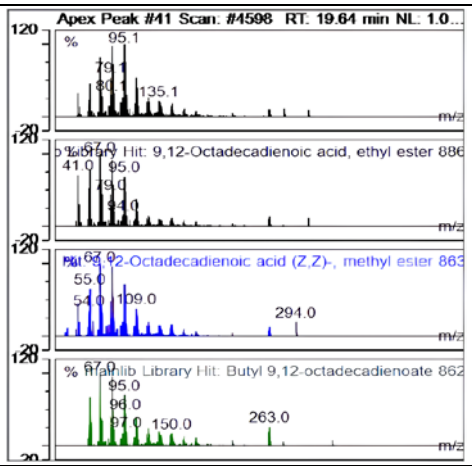
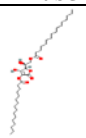
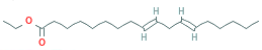
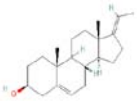
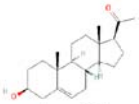
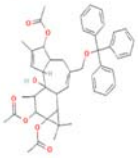
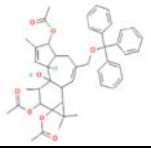


Fig. 4: Apex peak 9,12-octadecadienoic acid, ethyl ester

Table 2: Compounds with various retention times, peak area percentages, molecular formulas, and molecular weights appeared after GC-MS analysis of the ethanol extract of kebiul seed kernel

No	Name	RT* (Min)	Peak area (%)	Molekuler formula	Molecular weight	Structure (2D PubChem)	SI (NIST)
1	l-(+)-Ascorbic acid 2,6-dihexadecanoate	18,16	8,97	$C_{38}H_{68}O_8$	652		856
2	9,12-Octadecadienoic acid, ethyl ester	19,64	5,86	$C_{20}H_{36}O_2$	308		886
3	6-Octadecenoic acid	19,93	16,28	$C_{18}H_{34}O_2$	282		877
4	Pregna-5,17(20)-dien-3-ol, (3ß,17E)-	23,21	2,52	$C_{21}H_{32}O$	300		728
5	Pregnenolone	24,90	11,77	$C_{21}H_{32}O_2$	316		720
6	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]	25,82	11,21	$C_{28}H_{38}O_9$	518		662
7	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-	26,11	9,58	$C_{28}H_{38}O_9$	518		722

Ret. Time:	19,928
	Hit# 1
Component:	6-Octadecenoic acid
SI:	877
RSI:	894
Library:	mainlib
	Hit# 2
Component:	cis-13-Octadecenoic acid
SI:	876
RSI:	880
Library:	mainlib
	Hit# 3
Component:	cis-Vaccenic acid
SI:	876
RSI:	879

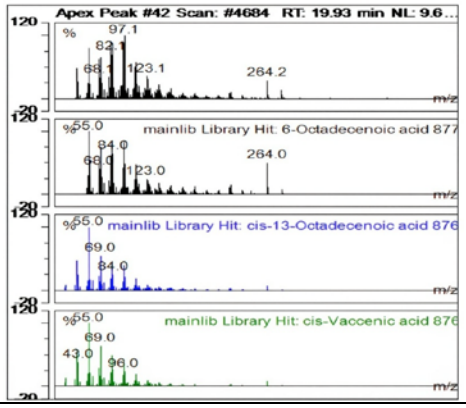


Fig. 5: Apex peak 6-octadecenoic acid

Ret. Time:	18,159
	Hit# 1
Component:	l-(+)-Ascorbic acid 2,6-dihexadecanoate
SI:	856
RSI:	856
Library:	mainlib
	Hit# 2
Component:	n-Hexadecanoic acid
SI:	830
RSI:	835
Library:	mainlib
	Hit# 3
Component:	Palmitic anhydride
SI:	818
RSI:	819

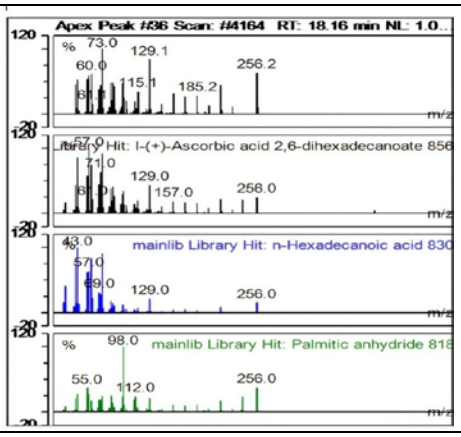


Fig. 6: Apex peak-(+)-ascorbic acid 2,6-dihexadecanoate

Ret. Time:	23,213
	Hit# 1
Component:	Pregna-5,17(20)-dien-3-ol, (3ß,17E)-
SI:	728
RSI:	758
Library:	mainlib
	Hit# 2
Component:	Retinoic acid
SI:	715
RSI:	735
Library:	mainlib
	Hit# 3
Component:	Androst-4-en-11-ol-3,17-dione, 9-thiocyanato-
SI:	709
RSI:	714
Library:	mainlib

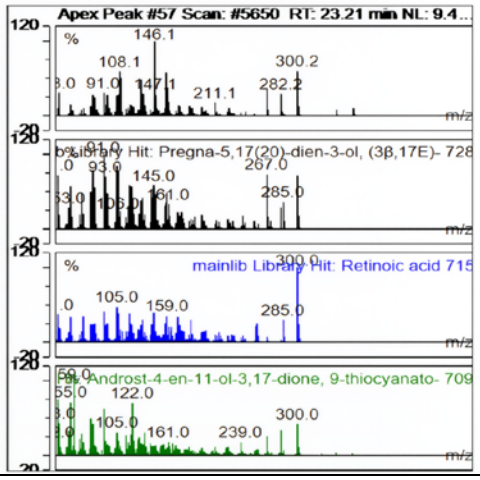


Fig. 7: Apex peak pregna-5,17(20)-dien-3-ol, (3ß,17E)-

Ret. Time:	26,108
	Hit# 1
Component:	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a, 5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß, 7aa,7ba,8a,9ß,9aa)]-
SI:	722
RSI:	722
Library:	mainlib
	Hit# 2
Component:	5,16,20-Pregnatriene-3beta,20-diol diacetate
SI:	702
RSI:	725
Library:	mainlib
	Hit# 3
Component:	17Alpha-ethynyl-17beta-hydroxy-6beta-methoxy-3alpha,5-cyclo-5alpha-androstan-19-oic acid
SI:	689
RSI:	739

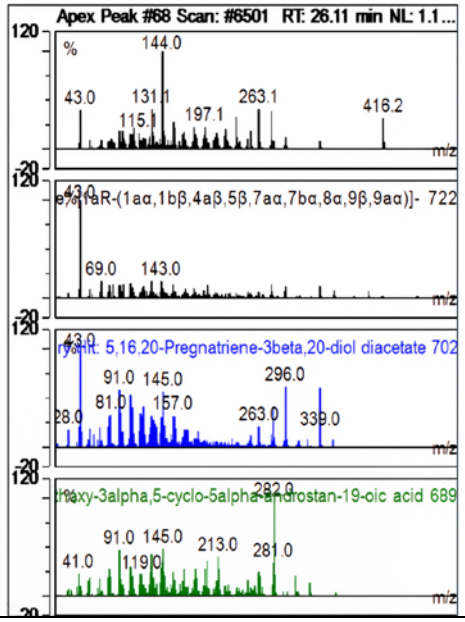


Fig. 8: Apek peak 1H-cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,3-[(acetyloxy)methyl]-1a,1b,4,4a,5, 7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a, 9ß,9aa)]-

Ret. Time:	24,897
	Hit# 1
Component:	Pregnenolone
SI:	720
RSI:	750
Library:	mainlib
	Hit# 2
Component:	6ß-Hydroxymethandienone
SI:	709
RSI:	747
Library:	mainlib
	Hit# 3
Component:	Bolasterone
SI:	708
RSI:	738

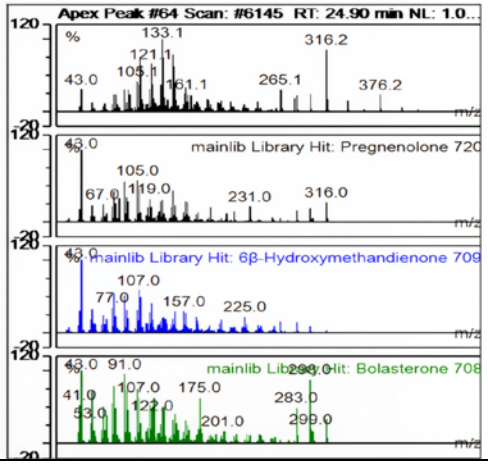


Fig. 9: Apek Peak pregnenolone

Ret. Time:	25,822
	Hit# 1
Component:	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5, 7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9, 9a-triacetate, [1aR-(1aa,1bß, 4aß,5ß,7aa,7ba,8a,9ß,9aa)]-
SI:	662
RSI:	663
Library:	mainlib
	Hit# 2
Component:	17Alpha-ethynyl-17beta-hydroxy-6beta-methoxy-3alpha,5-cyclo-5alpha-androstan-19-oic acid
SI:	645
RSI:	704
Library:	mainlib
	Hit# 3
Component:	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1aa,1bß, 4aa,5ß,7aa,7ba,8a,9ß,9aa)]-
SI:	642
RSI:	642
Library:	mainlib

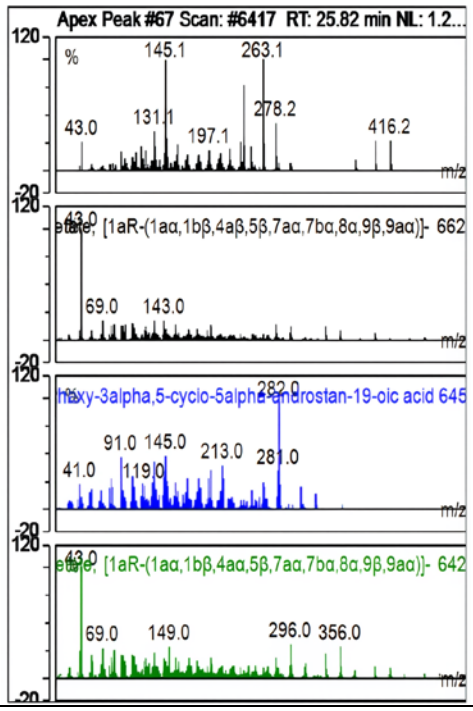


Fig. 10: Apek Peak1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,3-[(acetyloxy)methyl] 1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate, [1aR(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-

Table 3: Chromatogram of ethanol extract of kebiul seed coat by GC-MS.

No.	Ret. time min	Hit# 1	Chemical formula	Mol. weight	SI Hit#1	Rel. area %	Area counts*min
1	4,40	Butanoic acid, 4-butoxy-	$C_8H_{16}O_3$	160	690	0,65	17149251,603
2	4,90	1-(3,3,3-Trifluoro-2-hydroxypropyl)piperidine	$C_8H_{14}F_3NO$	197	678	1,98	52600361,831
3	5,46	Propanoic acid, 3-ethoxy-, ethyl ester	$C_7H_{14}O_3$	146	809	2,92	77439842,012
4	6,15	e-N-Formyl-L-lysine	$C_7H_{14}N_2O_3$	174	719	0,24	6348137,883
5	7,05	DL-Arabinose	$C_5H_{10}O_5$	150	665	2,72	72235304,066
6	7,52	Glycerin	$C_3H_8O_3$	92	834	5,19	137676830,908
7	7,97	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236	695	0,28	7424173,969
8	8,38	Acetamide, N-(2-acetyl-3-oxo-4-isoxazolidinyl)-	$C_7H_{10}N_2O_4$	186	687	1,75	46451880,017
9	9,37	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	718	0,26	6865816,496
10	9,89	6-Acetyl-ß-d-mannose	$C_8H_{14}O_7$	222	702	0,37	9807341,141
11	10,57	Ascaridole epoxide	$C_{10}H_{16}O_3$	184	720	0,20	5377155,199
12	11,10	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	689	0,26	6789871,482
13	11,41	d-Mannose	$C_6H_{12}O_6$	180	765	0,31	8239081,820
14	12,56	1,2,3-Benzenetriol	$C_6H_6O_3$	126	795	3,86	102304036,550
15	12,99	Melezitose	$C_{18}H_{32}O_{16}$	504	665	0,01	266193,201
16	13,40	Melezitose	$C_{18}H_{32}O_{16}$	504	747	1,07	28348426,477
17	13,58	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$	255	719	0,04	933308,843
18	13,75	Melezitose	$C_{18}H_{32}O_{16}$	504	724	0,10	2628173,803
19	14,81	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	718	0,46	12147900,801
20	15,02	Melezitose	$C_{18}H_{32}O_{16}$	504	751	1,94	51576313,489
21	15,28	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	695	2,44	64609697,162
22	15,89	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	733	2,64	70056482,665
23	16,35	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	725	8,01	212444222,592
24	16,41	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	731	0,81	21420479,779
25	16,56	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	714	4,69	124442347,240
26	16,75	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	707	1,02	27033533,752
27	16,90	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{19}H_{34}O_6$	358	737	0,08	2119033,345
28	17,05	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{19}H_{34}O_6$	358	726	0,38	10019404,547
29	17,37	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	824	0,51	13473523,905
30	17,96	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	875	6,45	171053124,069
31	18,82	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	822	0,36	9504109,889
32	19,00	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	909	0,99	26351755,956
33	19,65	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	886	33,68	893553961,822
34	19,81	Octadecanoic acid	$C_{18}H_{36}O_2$	284	895	3,51	93133812,412
35	21,13	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	800	0,31	8280821,105

No.	Ret. time min	Hit# 1	Chemical formula	Mol. weight	SI Hit#1	Rel. area %	Area counts*min
36	21,32	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	792	0,27	7034298,457
37	21,43	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	778	0,53	14171794,774
38	22,09	1,4-Naphthoquinone, 2-acetyl-5,8-dihydroxy-3-methoxy-	$C_{13}H_{10}O_6$	262	719	0,37	9832281,596
39	22,62	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	791	0,64	17030916,494
40	23,15	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	760	0,50	13224711,648
41	23,99	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	826	0,89	23739730,221
42	24,68	6ß-Hydroxymethandienone	$C_{20}H_{28}O_3$	316	737	1,64	43534942,669
43	25,32	7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-, 2,4,7,10,11-pentaacetate	$C_{30}H_{44}O_{11}$	580	745	0,28	7490606,831
44	25,56	1H-Cyclopropa[3,4]benz[1,2-e]azulene-3-carboxaldehyde, 9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-4a,7b,9-trihydroxy-1,1,6,8-tetramethyl-5-oxo-, [1aR-(1aa,1bß,4aß,7aa,7ba,8a,9ß,9aa)]-	$C_{22}H_{28}O_7$	404	613	1,64	43411487,584
45	27,82	Prednisolone Acetate	$C_{23}H_{30}O_6$	402	713	0,28	7344743,487
46	28,32	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	711	0,73	19360722,136
47	28,51	(+)--Tocopherol, O-methyl-	$C_{29}H_{50}O_2$	430	778	0,44	11561126,095
48	29,74	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	$C_{28}H_{40}O_4$	440	702	0,05	1402279,047
49	30,07	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	800	0,36	9572451,194
50	31,75	-Sitosterol	$C_{29}H_{50}O$	414	865	0,91	24167148,635

Table 4 summarizes the three compounds in the ethanol extract of the seed coat that have the highest relative peak areas (%) in this investigation, whereas fig. 12-14 summarizes the apex peak compound and real-time.

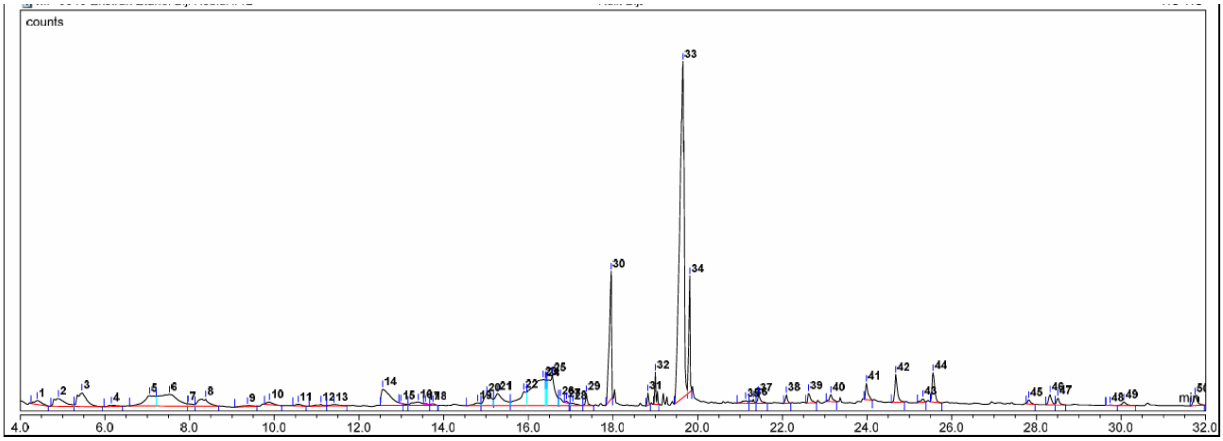
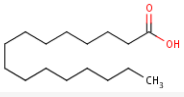
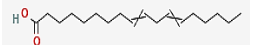
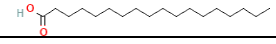


Fig. 11: Chromatogram of ethanol extract of kebiul seed coat by GC-MS

The chromatogram of the kebiul seed coat, as illustrated in fig. 11 and detailed in table 3, showcases the retention times and peaks identified through GC-MS analysis.

Fig. 11 presents the chromatogram obtained from the ethanol extract of the kebiul seed coat. Compounds were identified by comparing their mass spectra with a primary database, ensuring a Similarity Index (SI) exceeding 80% (NIST), and by cross-referencing with published literature values. GC-MS analysis identified 50 phytochemical compounds in the seed coat extract (table 3).

Table 4: Compounds with various retention times, percentage of peak area, molecular formula, and molecular weight appeared after GC-MS analysis of ethanol extract of kebiul seed coat

No	Name	RT* (Min)	Peak area (%)	Molekuler formula	Molecular weight	Structure	SI
1	n-Hexadecanoic acid	17,96	6,45	$C_{16}H_{32}O_2$	256		875
2	9,12-Octadecadienoic acid (Z,Z)-	19,65	33,68	$C_{18}H_{32}O_2$	280		886
3	Octadecanoic acid	19,81	3,51	$C_{18}H_{36}O_2$	284		895

Ret. Time:	17,955
	Hit# 1
Component:	n-Hexadecanoic acid
SI:	875
RSI:	880
Library:	mainlib
	Hit# 2
Component:	l-(+)-Ascorbic acid 2,6-dihexadecanoate
SI:	859
RSI:	859
Library:	mainlib
	Hit# 3
Component:	Palmitic anhydride
SI:	810
RSI:	810

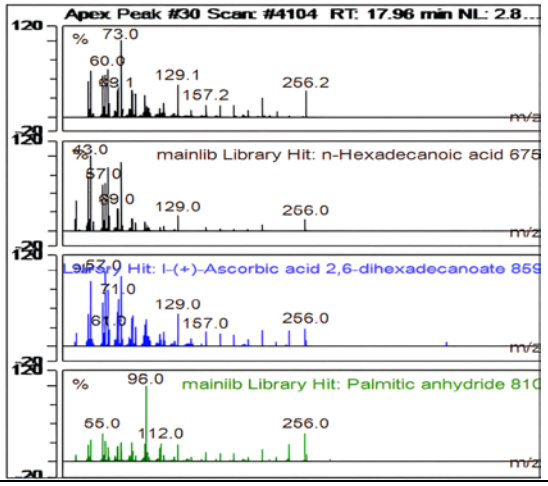


Fig. 12: Apex peak GC-MS hexadecanoic acid

Ret. Time:	19,652
	Hit# 1
Component:	9,12-Octadecadienoic acid (Z,Z)-
SI:	886
RSI:	886
Library:	mainlib
	Hit# 2
Component:	(Z)-18-Octadec-9-enolide
SI:	883
RSI:	896
Library:	mainlib
	Hit# 3
Component:	Linoelaidic acid
SI:	852
RSI:	922

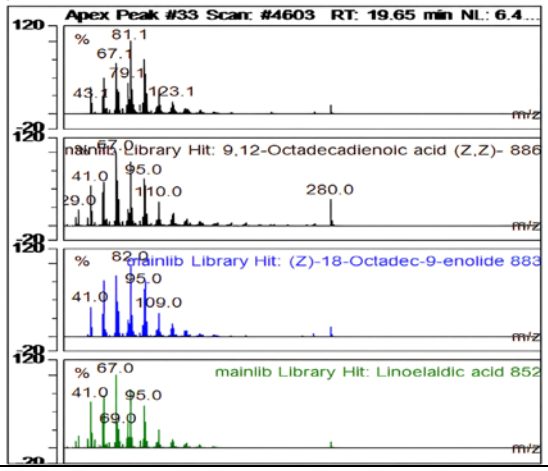


Fig. 13: Apek peak 9,12-octadecadienoic acid (Z,Z)-

Ret. time:	19,812
	Hit# 1
Component:	Octadecanoic acid
SI:	895
RSI:	899
Library:	mainlib
	Hit# 2
Component:	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester
SI:	817
RSI:	830
Library:	Mainlib
	Hit# 3
Component:	L-Ascorbic acid, 6-octadecanoate
SI:	769
RSI:	769

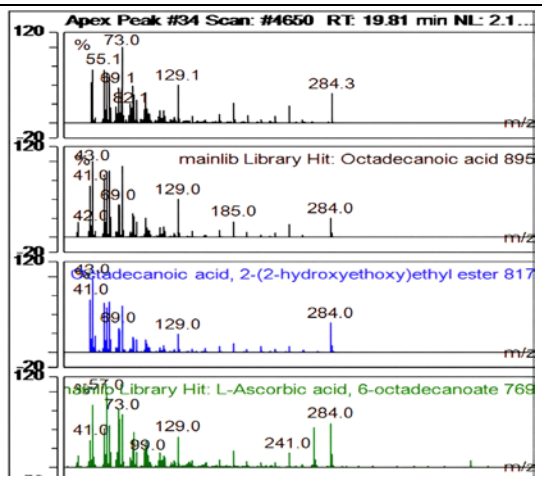


Fig. 14: Apex peak octadecanoic acid

In contrast to the main library, fig. 12-14 shows the compound's apex peak at a certain moment as well as its resemblance to other compounds.

DISCUSSION

The kingdom of plants contains an endless supply of active chemicals for medications. In the quest for resources and raw materials for the pharmaceutical sector, phytochemical approaches are crucial. Finding new drugs is a drawn-out process that includes several steps that must be taken one after the other. Fresh and dried plant materials can be extracted using various of methods, either with or without the use of solvents (expression, sublimation, distillation, etc). These methods may include the use of water, ether, acetone, methanol, ethanol, chloroform, etc. A key step in the

analysis of medicinal plants is isolation, which involves several basic procedures, including prewashing, drying or freeze-drying plant materials, grinding to produce a homogenous sample, and frequently enhancing the kinetics of analytic extraction and increasing sample surface contact with the solvent system. Qualitative assays for screening phytochemical compounds are the main way to start phytochemical characterization. The last stages in the photochemical study of plants are the characterization and identification of the isolated and separated constituents [10].

The selection of plant material, phytochemical analysis, characterization, and pharmacological investigation are all steps in the drug development process. A thorough preclinical inquiry is then conducted before clinical trials begin. Until 1996, naturally occurring chemicals either inspired or directly derived almost 80% of pharmaceuticals. Between 1981 and 2019, 1881 new medications were approved; of these, about 23.5% were natural products or semisynthetic derivatives of natural products, and about 25% were either pharmacophores or natural product mimics. The assessment of natural products in novel drug discovery has been revolutionized by the emergence of new skills [11].

By utilizing both mass spectra and retention periods, GC-MS is an effective method for compound identification. The GC-MS chromatogram examination of the ethanol extract revealed that the ethanol extract of seeds contained 87 bioactive phytochemical compounds (fig. 2), whereas the ethanol extract of the seed coat contained 50 bioactive phytochemical compounds (fig. 11). Tables 1-4 highlight the three compounds in the ethanol extract of the seed coat and the seven compounds in the ethanol extract of the seed kernel with the largest relative peak areas, as well as the apex peak compound and real time. The amount of time it takes for a chemical to move from the GC column to the detector is known as the retention time. It is an essential criterion for compound identification. When compared to the mainlib collection, the mass spectrum's apex peak similarity and retention duration are matched to different chemicals (fig. 4-13).

The bioactive chemicals from the coral Junceella delicata were separated and then processed for structure elucidation using GC-MS and FTIR. These substances include ethyl aminomethyl formimidate, Gly-Gly, and 2-(2-Pyridyl)-4-methylthiazole-5-carboxylic acid, 7-Methoxy-2-methylquinolin-4-ol, fraxidin, 2-methyl-3-trans-propenylpyrazine, 3-tert-butylpyridine, acetaldehyde benzyl ethyl acetal, α-methylcinnamicacid, 4-ethoxycoumarin, 3-hydroxycoumarin, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, 2,2-bis(3-allyl-4-hydroxyphenyl) propane, phenyltriethylammonium cation, and dodecanedioic acid [12]. Using spectroscopic data from the literature, five lignans (1–5), two phloroglucinols (6–7), five flavonoids (8–12), and four phenolics (13–16) were discovered from the leaves of *Caesalpinia bonduc*. The genus *Caesalpinia yielded* compounds 1–4, 6–7, 11, and 13–14, which are useful chemotaxonomic markers for *Caesalpinia bonduc* [13]. Determine secondary metabolites and analyze the methanol extract of *Caesalpinia bonduc* seeds using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) as well as antibiotic activity. Alkaloids, flavonoids, saponins, tannins, and phenolics are detected by phytochemical screening of the methanol extract from *Caesalpinia bonduc* seeds. Three major chemicals were identified by the GC-MS analysis: 4-Methyl-1,3-Dioxolan-2-One, 2-Trimethylsilyl-1,3-dithiane, and Cholesta-5,7,9(11)-trien-3-ol acetate. The FTIR study revealed that the extract contains the functional groups O-H, N-H, C-N, C-H, C-O, C=C, C=O, C-S, and C-Si. *Escherichia coli* and *Staphylococcus aureus* growth was inhibited by the antibacterial activity methanol extract from *Caesalpinia bonduc* seeds at different doses [14].

This work used gas chromatography and mass spectroscopy to identify the bioactive chemicals found in the ethanol extracts of kebiul seed kernel and kebiul seed coat. Retention time (RT), molecular formula, molecular weight (BM), and concentration (peak area%) are all active principles. An intriguing technique for determining the concentration of certain active ingredients in herbs used in the food, pharmaceutical, cosmetics, environmental, and forensic industries is the GC-MS method, which is employed to analyze the extracted materials [15]. This method analyzes a combination of chemical components by combining two analytical techniques into one. The components of the mixture are separated by gas chromatography, and each component is examined independently by mass spectroscopy.

Utilize HPLC and GC-MS methodologies to examine the bioactive constituents of Matricaria chamomilla (chamomile) flowers, encompassing flavonoids, terpenoids, and polyphenols [16]. 20 chemicals were identified as a result of the n-hexane extract's gas chromatography-mass spectrometry (GC-MS) investigation. These comprised siloxanes, fatty acid esters, sterols (e. g., stigmasterol and ergost-5-en-3-ol), terpenoids (e. g., phytol and caryophyllene oxide), alkanes, and other hydrocarbons. Numerous substances, such as erucamide, phytol, and squalene, have been investigated for their known antibacterial and anticancer properties.

Caesalpinia bonduc is utilized in ointments including castor oil and seed powder, which can be applied topically to address hydrocele and orchitis [17]. Seed-derived oil can be applied topically to address convulsions and paralysis. The consumption of equal quantities of pepper and *Caesalpinia bonduc* seed powder has demonstrated antimalarial properties [18]. They utilized such grains as necklaces [19]. Seed oil moisturizes the skin and alleviates acne. *Caesalpinia bonduc* seeds, as a polyherbal formulation, are utilized in the treatment of diphtheria (7 g of mixed seeds) and pneumonia (5 g of mixed seeds), as indicated by an ethnobotanical study [20].

The seeds are utilized for several medicinal conditions, including hemostatic, laxative, anthelmintic, inflammation, colic, malaria, skin disorders, and leprosy, according to Singh and Raghav (2012). The seeds' extracts are also utilized as anthelmintic and anti-blennorrhagic medicines, and they are also used as tonics [22]. Traditionally, the seed extract is used with castor oil or honey to make this anthelmintic medication [23]. Traditional folk pre-treat intermittent fever with *Caesalpinia bonduc* juice for two weeks. To treat hydrocele and orchitis topically, seed powder has also been combined with castor oil to create an ointment [24]. According to a recent assessment by Sasidharan *et al.* (2021), the best method of treating health issues is to take roasted and powdered seeds orally. Similarly, a review examined the potential of *Caesalpinia bonduc* in treating polycystic ovarian syndrome (PCOS) [2]. To energize the body and ease pain, the seeds are roasted and ground into a powder. The seeds, which are made as a poultice with asafoetida, ghee, and salt to taste, are also used to treat postpartum stomach pain.

Colitis, diarrhea, and dysentery have all been treated using the seed coat. Amra, Haridra, and Palasa can be taken with roasted seed powder or leaf juice to treat worm infestation-induced irritation in the anal area. In India, the roasted seeds are marketed as Latakaranja after being mixed 1:1 with pippali. An approved herbal treatment for malaria, it can be administered at intervals of 0.5 g per day for 3–4 d [26]. The extract of this plant is an excellent treatment for an enlarged spleen caused by malaria [27]. Additionally, they reported that the plant's seeds are used to treat menstrual pain in the abdomen, boost menstrual flow during oligomenorrhea, and enhance uterine function. Because the seed coat is so effective at treating fluoride, it is utilized to absorb the fluoride present in drinking water. Therefore, the first step in identifying the active chemicals in this plant is to analyze the ethanol extract of the seed kernel and seed coat of kebiul using GC-MS.

CONCLUSION

GC-MS analysis revealed the ethanol extract of the kebiul seed coat included 50 phytochemical compounds, with the highest compound at a retention time of 19.65 min, namely 9,12-Octadecadienoic acid (Z,Z)-. Subsequently, n-Hexadecanoic acid exhibits a retention time of 17.96 min, while Octadecanoic acid has a retention time of 19.81 min. While the ethanol extract of the kebiul seed kernel contained 87 phytochemical compounds. Since these results are preliminary and only based on chemical identification, more investigation is required, with the highest compound at a retention time of 19.93 min, namely 6-Octadecenoic acid. Subsequently Pregnenoloneexhibits a retention time of 24.90 min, 1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-

tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-exhibits a retention time of 25.82 min,1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-has a retention time of 23.21 min, l-(+)-Ascorbic acid 2,6-dihexadecanoate has a retention time of 18.16 min, and 9,12-Octadecadienoic acid, ethyl ester has a retention time of 19.64 min, and Pregna-5,17(20)-dien-3-ol, (3ß,17E)-has a retention time of 23.21 min.

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AUTHORS CONTRIBUTIONS

Densi Selpia Sopianti: Conceptualization, Writing–Original Draft; Muchsin Doewes: Writing the Original Draft and conducting the Review and Editing; Tatar Sumandjar: Writing–Original Draft, Writing–Review and Editing; Eti Poncorini Pamungkasari: Methodology, Writing–Original Draft; Paramasari Dirgahayu: Methodology, Writing–Original Draft; Ratih Puspita Febrinasari: Methodology, Writing–Original Draft.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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