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Gas Chromatography-Mass Spectrometry Analysis of Ethanol Extract *Etlingera elatior* Fruit

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ABSTRACT

Etlingera elatior known locally as Patikala in Indonesia is a local plant known to contain bioactive compounds, including acidic compounds that play a crucial role in various biological activities. This study aimed to identify and characterize acidic compounds contained in patikala fruit extracts using Gas Chromatography-Mass.Spectrometry (GC-MS). Patikala fruits were extracted by maceration in 96% ethanol. The bioactive compounds profile, especially the organic acids profile of patikala fruit ethanol extract was determined by GC-MS analysis. Forty (40) acidic compounds and their derivatives were identified in the GC-MS analysis of the ethanol extract of patikala fruit. Ten acidic compounds, including 9-Octadecenoic acid (Z)-, methyl ester ($C_{19}H_{36}O_2$), Propanoic acid, 3-mercapto-, dodecyl ester ($C_{15}H_{30}O_2S$), 1-(2-fluoro-phenyl)-5-oxo-pyrrolidine-3-carboxylic acid phenyl ($C_{17}H_{15}FN_2O_2$), Carbonic acid, octyl 2.2.2-trichloroethyl ester ($C_{11}H_{19}Cl_3O_3$), Alpha.-[2.5-dimethyl-3-thienyl]-.beta.-[p-bromophenyl] acrylic acid ($C_{15}H_{13}BrO_2S$), Succinic acid, cyclohexylmethyl non-3-en-1-yl ester ($C_{20}H_{34}O_4$), Cyclopropanedecanoic acid, 2-hexyl-.alpha.-hydroxy-, methyl ester ($C_{20}H_{38}O_3$), 9-Octadecenoicacid (Z)-, 3-[(1-oxohexadecyl)oxy]-2-[(1-oxooctadecyl)oxy] propyl ester ($C_{55}H_{104}O_6$), 9-Octadecenoic acid (Z)-, octyl ester ($C_{26}H_{50}O_2$), and 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester ($C_{37}H_{70}O_5$) were identified directly. Cyclopropanedecanoic acid was identified as the major compound with a percentage area of 1.49%. This carboxylic acid, characterized by a long carbon chain and a cyclopropane ring, has potential for development in polymer research that utilizes organic compounds as functional components.

Keywords: Acidic Compounds, Ethanol Extract, Maceration, *Etlingera Elatior* (Patikala)

Introduction

Indonesia is rich in natural resources, including a variety of spices and local plants. One of the local plants is *Etlingera elatior*, locally known as the patikala fruit. It is known by different local names, including "patikala" in South Sulawesi, "Sambuang" in West Sumatra, "Kincung" in North Sumatra, "Kecombrang" in Java, "Honje" in Sunda, "Katan" in Kalimantan, and "Bongkot" in Bali. The patikala plant (*Etlingera elatior*), a member of the *Zingiberaceae* family,[1,2] is commonly known as torch ginger due to its flower shape resembling a torch and its distinctive red colour.[3] The plant is also called red ginger lily, and it grows in tropical countries, almost throughout the mainland of Southeast Asia.[4] The patikala plant has long history of use as both a culinary spice and a herbal medicine, with nearly all parts, including the rhizomes, stems, leaves, flowers, and fruits, valued for their rich composition of bioactive compounds, particularly flavonoids, phenols, tannins, and a diverse range of other secondary metabolites. The phenolic content in its ethanol extract has been reported as 3.76% in rhizomes, 6.66% in leaves, and 5.67% in flowers.[5] The leaf extract also contained flavonoid (10.77% w/w) and phenol (3.75% w/w).[6]

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Furthermore, phenolic acid derivatives such as gallic acid (129.14 mg/100 g DM), tannic acid (82.66 mg/100 g DM), chlorogenic acid (75.70 mg/100 g DM), and caffeic acid (88.46 mg/100 g DM) have been identified in *Etlingera elatior* flowers from Kelantan.[7] However studies on acidic compounds in fruit are still limited, hence the need for further research in this direction. The acidic compounds in patikala fruit can be extracted using maceration,[8] a process that separates a mixture of materials using a suitable solvent.[9] Maceration is a conventional method, involving a simple extraction process using a solvent with frequent stirrings at room temperature.[10,11] The maceration extraction method ensures that the active substance is not damaged or decomposed, it is fast, and relatively cheap.[12,13] Besides, it is important to consider the type of solvent used for maceration in order to optimize the extraction process.[14] An organic solvent is necessary to dissolve the compound most effectively in maceration extraction.[15] In this study, the extraction of *Etlingera elatior* fruit used ethanol as a solvent due to its positive properties, including safety, availability, and affordability. In addition, ethanol also exhibits semi-polar properties, which enable the dissolution of both polar and non-polar compounds from Simplicia.[16] Compounds in the extracts can be detected by gas chromatography and mass spectrometry (GC-MS) analysis.[17] This tool identifies compounds either organic or inorganic in samples based on their retention time and molecular weight.[18] The advantage of this method is its ease of use, and high sensitivity in detecting volatile compounds, and profiling of more complex extracts.[19] GC-MS data provide essential information regarding the volatile, non-ionic, and thermally stable components of extracts, and can detect relatively low molecular weight compounds.[20] This study, therefore aimed to obtain more in-depth information on the acidic compounds in patikala fruit by employing GC-MS tool.

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Materials and Methods

Solvent and Equipment

The materials used include 96% ethanol (Merk Indonesia), a polyethylene plastic (Merk Indonesia), a rotary vacuum evaporator (Model RE-1000HN (Horizontal), China), and a GC-MS instruments (QP2010 Plus, Japan).

Plant collection and identification

The pink coloured patikala (*Etlingera elatior*) fruits with black seeds were collected from Lumaring Village, Larompong District, Luwu Regency, South Sulawesi Province, Indonesia (3°30′48.71″S 120°20′5.60″E) in February 2024. The plant was identified at the Chemical Engineering Laboratory, Ujung Pandang State Polytechnic, Hasanuddin University South Sulawesi, Indonesia where a voucher specimen was kept with the voucher number 01/AR/OC/2024.

The fruits were peeled and washed with running water, then drained for approximately 15 minutes. Subsequently, the fruits were pressed until soft and placed in polyethylene plastic bags until further analysis.

Extraction of plant material

The softened fruits were extracted using the maceration method. Approximately 500 g of chopped patikala fruits in a glass jar was macerated in 1000 mL of 96% ethanol at room temperature (25°C) for 24 h.[21-23] The macerate was filtered, and the filtrate was concentrated *in vacuo* using a rotary evaporator at 50°C. The extract was then placed in a vial and flushed with nitrogen gas, at which point it was ready for analysis.

GC-MS analysis

GC-MS analysis was done according to standard procedure with a GC-MS spectrometer (GCMS_QP2010, Shimadzu, Japan), which consists of an auto-sampler (AOC-20i) and a Gas Chromatograph (GC-2010 Plus) connected to a Mass Spectrometer. A total of 5 mL of the sample was manually injected in a splitless mode. The injector temperature was maintained at 260°C, detector temperature at 250°C, and column temperature at 325°C. The ionization was by electron impact in positive mode at an ionization energy of 70 eV. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The transfer line temperature was set at 200°C and increased to 240°C. The oven temperature was programmed to start from 70°C and increased to 220°C at a rate of 10°C/minute, held for 1 minute, and then increase to 300°C. The total run time was 35 minutes.[24,25] The data obtained included the compound name, molecular formula, peak area, total area, and peak height. Compounds were identified by matching the mass spectrum of each chromatogram peak with the National Institute of Standards and Technology (NIST) database.[26,27]

Results and Discussion

The study identified the phytoconstituents of patikala fruit ethanol extract by GC-MS analysis. GC-MS is a highly sensitive tool for detecting volatile, non-ionic, and thermally stable components of extracts. Figure 1 shows the gas chromatogram of the ethanol extract of patikala fruits. In GC-MS analysis, components of extract are separated into several peaks, each peak corresponding to a compound, which is then identified based on the mass spectra data.[28] In this study, forty (40) organic acids and their derivatives were successfully identified from the ethanol extract of patikala fruit by GC-MS analysis (Table 1). Ten (10) of the compounds were identified directly as organic acids, while thirty (30) were identified as acid derivatives. The ten directly identified organic acids were 9-Octadecanoic acid (Z), methyl ester (peak 1, 0.48%, ret. time = 3.76 min), Propanoic acid, 3-mercapto-,dodecyl ester (peak 10, 1.05%, ret. time = 7.89 min), 1-(2-fluoro-phenyl)-5-oxo-pyrrolidine-3-carboxylic acid phenyl (peak 13, 1.38%, ret. time 16.07 min), Carbonic acid, octyl 2.2.2 -trichloroethyl ester (peak 15, 1.27%, ret. time 20.23 min), Alpha.-(2.5-dimethyl-3-thienyl)-.beta.-(p-bromophenyl) acrylic acid (peak 16, 1.07%, ret. time = 20.67 min), Succinic acid, cylohexylmethyl non-3-en-1-yl ester (peak 17, 1.28%, ret. time = 22.33 min), Cyclopropanedecanoic.acid, 2-hexyl-alpha-hydroxy,.methyl ester (peak 35, 1.49%, ret. time 41.85 min), 9-Octadecanoic acid (Z)-, 3-[(1-oxohexadecyl]-2[(1-oxooctadecyl)oxy]propyl ester (peak 37, 1.33% ret. time = 42.99 min), 9-Octadecenoic acid (Z)-, octyl ester (peak 38, 1.43%, ret. time = 43.17 min), and 9-Octadecanoic acid(Z)-, 2-hydoxy-3-[(1-oxohexadecyl)oxy]propyl ester (peak 40, 0.41% ret. time 43.43 min).

The molecular formulas and structures of the acidic compounds in the ethanol extract of patikala fruits are shown in Table 2.

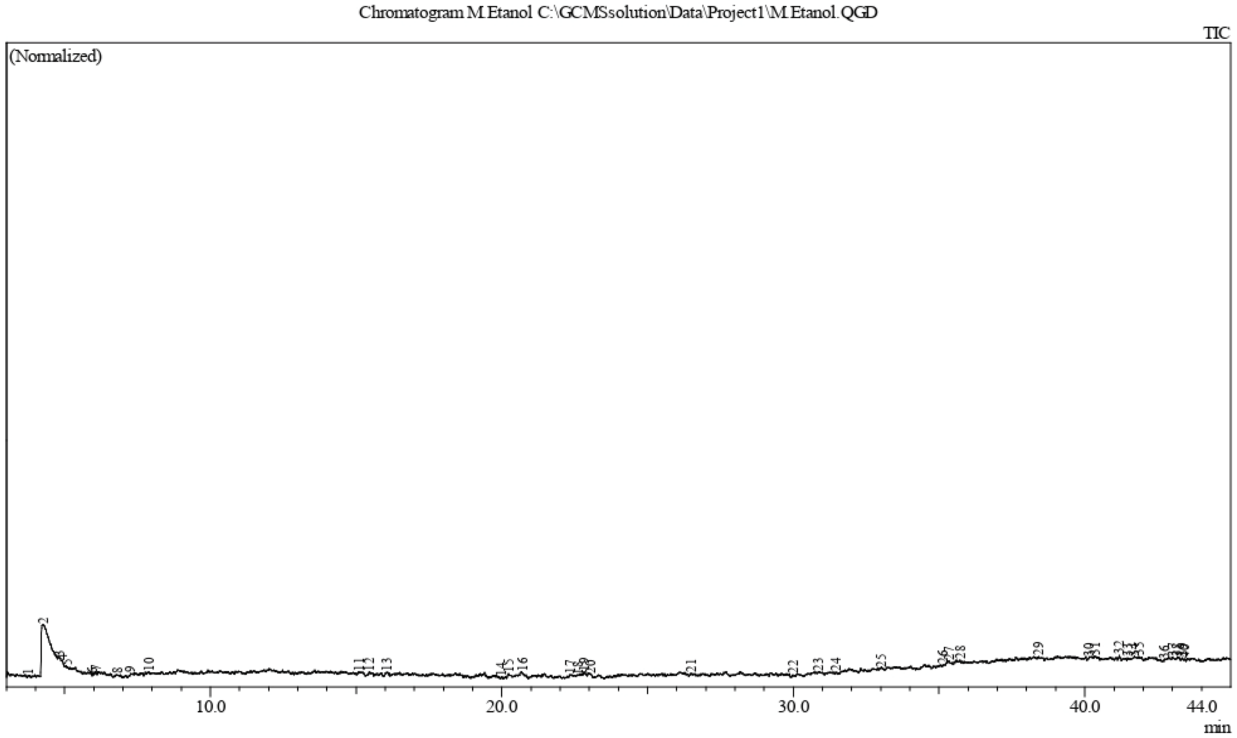

Figure 1: GC-MS Chromatogram of ethanol extract of Patikala (*Etlingera elatior*) fruit

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Table 1: Compounds identified from the GC-MS analysis of the ethanol extract of Patikala (*Etlingera elatior*) fruit

Peak No	Retention Time (Min)	Compound Name	Molecular Weight	Molecular Formula	Area %
1	3,76	9-Octadecenoic acid (Z)-, methyl ester	296	$C_{19}H_{36}O_2$	0.48
2	4,26	2,5-Furandione, 3-methyl- (CAS)	112	$C_5H_4O_3$	53.89
3	4,83	Decane	142	$C_{10}H_{22}$	5.51
4	4,92	(Z,E)-(+-)-4-Hydroxymethyl-.alpha.,alpha.,8-trimethyl-3,7-cyclodecadienemathanol	238	$C_{15}H_{26}O_2$	1.83
5	5,05	4H-1,2,4-Triazole-3-thiol, 4-(2-chlorophenyl)-5-(1-methylethyl)-	253	$C_{11}H_{12}ClN_3S$	0.96
6	5,97	6-Thiadodecane, 1-[1-cycloazapropyl]-	229	$C_{13}H_{27}NS$	0.36
7	6,08	5.alpha.-Androstan-17.beta.-ol-3-one, pentafluoropropionate	436	$C_{22}H_{29}F_5O_3$	0.43
8	6,78	L-Proline, 1-[N-(5-oxo-L-prolyl)-L-histidyl]-, methyl ester (CAS)	377	$C_{17}H_{23}N_5O_5$	1.01
9	7,27	Neoclovene oxide	220	$C_{15}H_{24}O$	0.72
10	7,89	Propanoic acid, 3-mercapto-, dodecyl ester	274	$C_{15}H_{30}O_2S$	1.05
11	15,09	D-Glucofuranose, pentaacetate	390	C16H22O11	1.89
12	15,46	6-Chloro-4-phenyl-quinazoline 3-oxide	256	$C_{14}H_9ClN_2O$	1.16
13	16,07	1-(2-fluoro-phenyl)-5-oxo-pyrrolidine-3-carboxylic acid phenyl	298	$C_{17}H_{15}FN_2O_2$	1.38
14	19,98	(2s,5r)-2-(t-butyl)-5-methyl-4-oxo-1,3-dioxolan-5-yl acetaldehyde	200	$C_{10}H_{16}O_4$	0.6
15	20,23	Carbonic acid, octyl 2,2,2-trichloroethyl ester	304	$C_{11}H_{19}Cl_3O_3$	1.27
16	20,68	alpha.-[2,5-Dimethyl-3-thienyl]-.beta.-[p-bromophenyl]acrylic acid	336	$C_{15}H_{13}BrO_2S$	1.07
17	22,33	Succinic acid, cyclohexylmethyl non-3-en-1-yl ester	338	$C_{20}H_{34}O_4$	1.28
18	22,56	N-tert-butyl-n'-[4-(chloro-difluoro-methoxy)-phenyl]-6-morpho	428	$C_{18}H_{23}ClF_{26}O_2$	1.6
19	22,82	1,1'-Biphenyl, 2,2',3,3',4,5,5',6,6'-nonachloro-	460	$C_{12}HCl_9$	1.63
20	23,06	2-n-Butyl-6,7-dichloro-1H-benz[de]isoquinoline-1,3(2H)-dione	321	$C_{16}H_{13}Cl_2NO_2$	1.58
21	26,48	(7E,9E)-Heptadeca-7,9-dien-11,13-diyn-6-one	242	$C_{17}H_{22}O$	1.14
22	29,98	Longicamphor	221	$C_{15}H_{23}DO$	0.47
23	30,84	(1RS,2SR)-Methyl- 2,7,7-Trimethyl-3-oxobicyclo[3.3.0]oct-4-en-2-acetate	236	$C_{14}H_{20}O_3$	1.12
24	31,48	12,13-epoxy-3alpha,7alpha,15-trihydroxytrichothec-9-en-8-one m	338	$C_{17}H_{22}O_7$	0.38
25	33	6,15-Dibutyl-2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene	472	$C_{28}H_{40}O_6$	0.76
26	35,15	Carbromal (+ some breakdown)	236	$C_7H_{13}BrN_2O_2$	0.4
27	35,34	3,3-Diethoxy-1,1,1,5,5,5-hexamethyltrisiloxane	296	$C_{10}H_{28}O_4Si_3$	1.54
28	35,71	2-tert-butyl-4-methyl-6-(1-phenylethyl) phenol	268	$C_{19}H_{24}O$	1.64
29	38,42	3,17di(heptafluorobutyryl)-17.beta.-nortestosterone	666	$C_{26}H_{24}F_{14}O_4$	1.2
30	40,15	1.alpha.-(Hydroxymethyl)-7.alpha.,8.alpha.-dimethyl-7-(2-(3-furyl)ethyl)bicyclo[4.4.0]d	346	$C_{21}H_{30}O_4$	0.88
31	40,33	Cholest-4-en-3-one, 26-(acetyloxy)-	442	$C_{29}H_{46}O_3$	0.59
32	41,18	2,2,3,5,6,6,7-Heptamethyl[1,4,2,3,5,6,7] dioxapentasilepane	280	$C_7H_{24}O_2Si_5$	0.86
33	41,47	1(3ah)-pentalenone, 4,5,6,6a-tetrahydro-3a,6,6a-trimethyl-, (3a.a.alpha	164	$C_{11}H_{16}O$	1.01
34	41,68	exo-6-Chloro-3-(5-ethoxy-2-phenyloxazol-3-yl)benzopyran-4-one	371	$C_{20}H_{18}ClNO_4$	1.46

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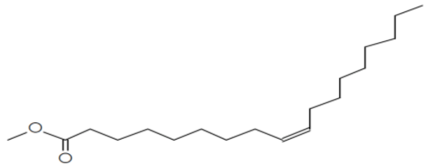
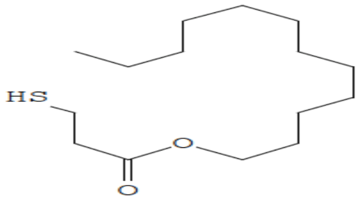
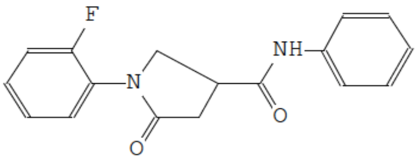
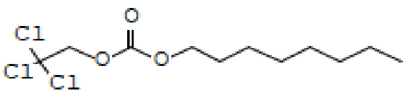
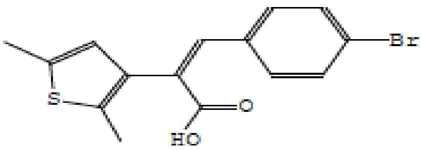
35	41,85	Cyclopropanedecanoic acid, 2-hexyl-.alpha.-hydroxy-, methyl ester	326	$C_{20}H_{38}O_3$	1.49
36	42,73	Butanoyl - anthracene	264	$C_{19}H_{20}O$	1.64
37	42,99	9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl)oxy]-2-[(1-oxooctadecyl)oxy]propyl ester	860	$C_{55}H_{104}O_6$	1.33
38	43,17	9-Octadecenoic acid (Z)-, octyl ester	394	$C_{26}H_{50}O_2$	1.43
39	43,34	6,6,10,10-Tetramethyl-1-thiaspiro[4.5]decane-3,3,4,4-tetracarbonitrile	312	$C_{17}H_{20}N_4S$	0.53
40	43,43	3.27 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	594	$C_{37}H_{70}O_5$	0.41

Table 2: Structure of ten (10) acidic compounds identified from the GC-MS analysis of the ethanol extract of Patikala (*Etlingera elatior*) fruit

No.	Compound Structure and Molecular Formula	Compound Name
1.	 $C_{19}H_{36}O_2$	9-Octadecanoic acid (Z), methyl ester
2.	 $C_{15}H_{30}O_2S$	Propanoic acid, 3-mercapto-, dodecyl ester
3.	 $C_{17}H_{15}FN_2O_2$	1-(2-fluoro-phenyl)-5-oxo-pyrrolidine-3-carboxylic acid phenyl
4.	 $C_{11}H_{19}Cl_3O_3$	Carbonic acid, octyl 2.2.2 -trichloroethyl ester
5.	 $C_{15}H_{13}BrO_2S$	Alpha.-(2.5-dimethyl-3-thienyl)-.beta.-(p-bromophenyl) acrylic acid

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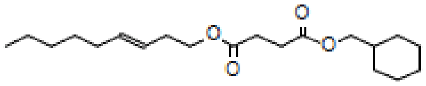
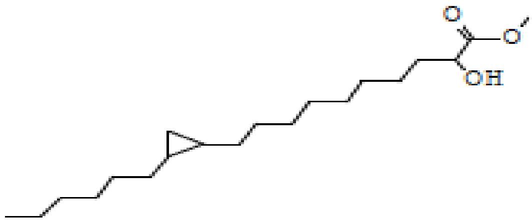
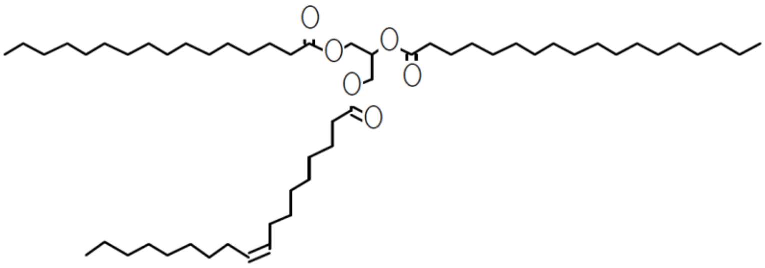
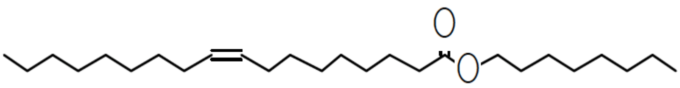
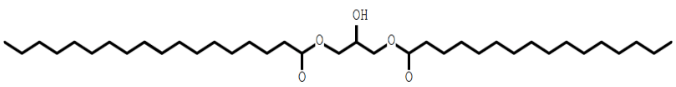
ISSN 2616-0692 (Electronic)

6.	 $C_{20}H_{34}O_4$	Succinic acid, cylohexylmethyl non-3-en-1-yl ester
7.	 $C_{20}H_{38}O_3$	Cyclopropanedecanoic acid, 2-hexyl-alpha-hydroxy,.methyl ester
8.	 $C_{55}H_{104}O_6$	9-Octadecanoic acid (Z)-, 3-[(1-oxohexadecyl]-2[(1-oxooctadecyl)oxy]propyl ester
9.	 $C_{26}H_{50}O_2$	9-Octadecenoic acid (Z)-, octyl ester
10.	 $C_{37}H_{70}O_5$	9-Octadecanoic acid(Z)-, 2-hydoxy-3-[(1-oxohexadecyl)oxy]propyl ester

Cyclopropanedecanoic acid, 2-hexyl-alpha-hydroxy-methyl ester ($C_{20}H_{38}O_3$) was found to be the most abundant acidic compound of the ethanol extract of patikala fruit with area percentage of 1.49%. The mass spectrum of this compound is presented in Figure 2. According to the National Institute of Standards and Technology (NIST), Cyclopropanedecanoic acid, 2-hexyl-alpha-hydroxy-methyl ester, is classified as an acidic compound with good solubility in ethanol. Ethanol is used as a solvent because it can extract compounds with various levels of polarity, both polar and non-polar compounds.[27,29] The two groups in ethanol, the polar hydroxyl group (OH) and the non-polar alkyl group (-R), allow such variable extraction.[30] Cyclopropanedecanoic acid, 2-hexyl-alpha-hydroxy-, methyl ester, like most carboxylic acids, is generally classified as a weak acid which are only partially ionized in aqueous solution. This weak acidity is due to the ability of such acids to donate protons (H^+) in solution, but with a low degree of ionization. In this case, cyclopropane decanoic acid has a carboxylic group (-COOH) responsible for its acidity, which remains weak, as with other carboxylic acids. It is a carboxylic acid with a long carbon chain and a cyclopropane ring. This compound can be developed for use as organic components in specific types of polymers.

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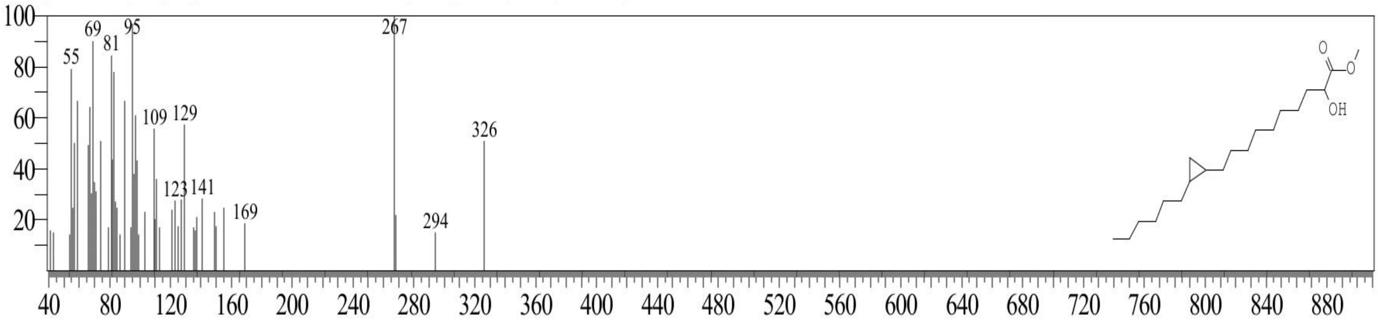

Figure 2: Mass spectrum of cyclopropanedecanoic acid, 2-hexyl-α.-hydroxy-, methyl ester ($C_{20}H_{38}O_3$) a major component of the ethanol extract of Patikala fruit

Conclusion

The GC-MS analysis of the ethanol extract of patikala fruits identified 40 organic acids, with cyclopropanedecanoic acid, 2-hexyl-alpha-hydroxy-methyl ester as the most dominant compound, accounting for 1.49% of the total compounds identified. This compound has been shown to have potential application as organic-based polymer.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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