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Research Article

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Bioactive Compounds in Methanolic Fruit Extract of *Garuga pinnata* Roxb

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ABSTRACT

Garuga pinnata (Roxb.) is one of the plant species reported from Palghar district of Maharashtra, belongs to the family Burseraceae with ethno-botanical values and is well-known for their Ethno-medicinal applications. It is commonly known as "Kakad" (Marathi). The present investigation was carried out to characterize the bioactive compounds present in methanolic fruit extract of Garuga pinnata using Gas Chromatography-Mass Spectrum (GC-MS) method. The results of GC-MS analysis provide different peaks determining the presence of 25 bioactive compounds. The major bioactive compounds 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5-Hydroxymethylfurfural, 1,2,3-Benzenetriol, beta-D-Glucopyranose, 1,6-anhydro, Quinic acid, Butanoic acid, octyl ester, n-Hexadecanoic acid and cis-13,16-Docasadienoic acid and minor compounds were also present. Majority of the compounds were belonging to acid group. Hitherto no reports exist on the Phytochemical analysis of Garuga pinnata (Roxb).

INTRODUCTION

Over the last few decades, use of herbal drugs has been emphasized due to their easy availability, therapeutic potential, least side effects and minimum cost. At present nearly 80% of the world population rely on plant based drugs for their health care need. Various plants still available in the nature are yet to be explores for their medicinal potential. (Gosh *et al*. 2015). In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key

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technological platform for secondary metabolite profiling in plant species.

Garuga pinnata Roxb. (Family: Burseraceae) is a deciduous tree reaching 50ft in height, with bark pealing off in flakes. Leaves are Pinnate, 6-18 in. long with 6-10 pairs of leaflets, opposite. Flowers are small, polygamous, creamy white or yellow, 4 mm across, in axillary panicles to 15 cm; bracts linear to 2 mm; calyx campanulate, tomentose, deciduous; lobes 5, ovate; petals 5, oblong-lanceolate, tomentose, thickened and inflexed at apex; disc crenate, yellow; stamens 10, inserted on calyx tube; filaments 1.5 and 2.5 mm, pubescent; ovary superior, globose, pilose, 5-celled, ovules 2 in each cell; style pubescent; stigma 5-lobed. Fruits a drupe, oblong or irregularly globose, greenish-yellow. The fruits are eaten raw or pickled.

A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide, related to the possible chemical components of "*Garuga pinnata"* Roxb. So, the present study was aimed to investigate the possible bioactive compounds by first preparing the methanolic extract and identification of the compounds by subjecting it to GC-MS analysis.

Photo plate 1: Fruits of *Garuga pinnata* Roxb.

Photo plate 2: Flowers of *Garuga pinnata* Roxb.

MATERIALS AND METHODS

Collection of Plant Material:

The fully matured Fruits of *Garuga pinnata* (Roxb.) were collected from Palghar district, Maharashtra, India. A Voucher specimen was deposited in the herbarium of Department of Botany, Sonopant Dandekar College, Palghar.

Preparation of Plant extract:

The Fruits of *Garuga pinnata* were used for preparation of extract. Five gram of oven dried powder were taken in a 250 ml flask and mixed thoroughly with 50 ml of 100% methanol (pure methanol). Methanolic extract were obtained in an electric shaker (Remi Rotary Shaker, Mumbai, India) for 48 hour in ambient condition (shaking intensity 120 rpm). The extract were then filtered using Whatman No.1 filter paper. Filtrate is then concentrated till dry residue was remained. After weighing the residue, respected amount of methanol was added to make the final solution. This solution were further used for GC-MS for analysis.

Photo plate 4: Filtration process

Photo plate 3: Fruit Powder of *G. pinnata*

Photo plate 5: Final solution

GC-MS analysis of bioactive compounds from wild edible plants:

The methanolic extract obtained from fruit of *Garuga pinnata* were subjected to Gas chromatography- Mass Spectroscopy for the determination of bioactive volatile compounds. Some of the important features are summarized below.

GC-MS analysis of the sample were carried out using Shimadzu Make QP-2010 with non-polar 60 M RTX 5MS column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40^0C and held for 3 min and the final temperature of the oven was 48^0C with rate at 10^0C [Min.sup.-1]. A 2 µL sample was injected with split less mode. Mass spectra was recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample is 45 min. The chemical components from the methanolic extract plant were identified by comparing the retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

RESULTS AND DISCUSSION

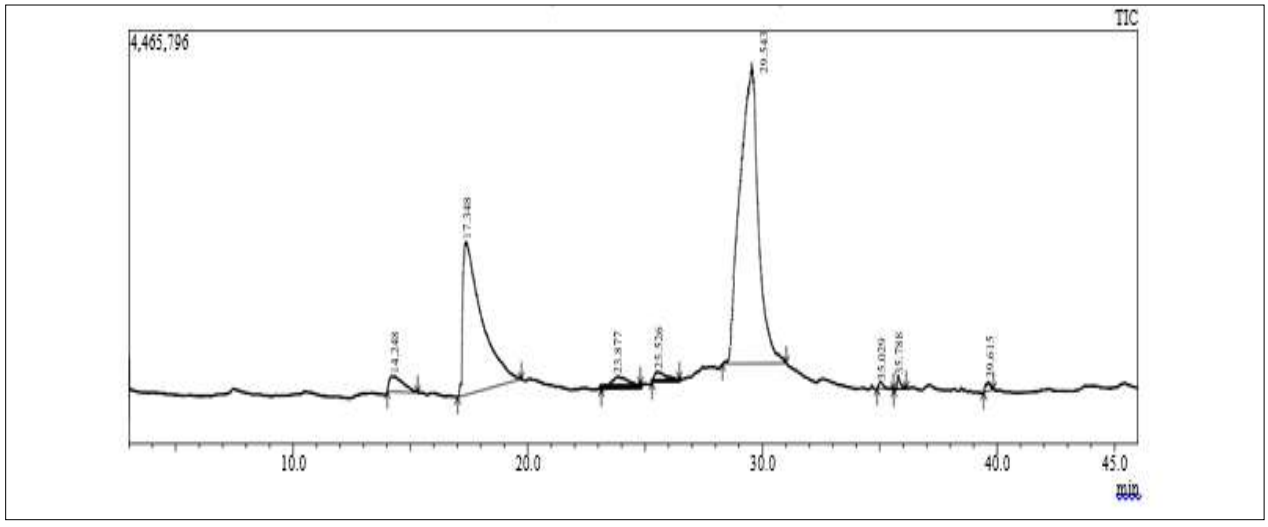


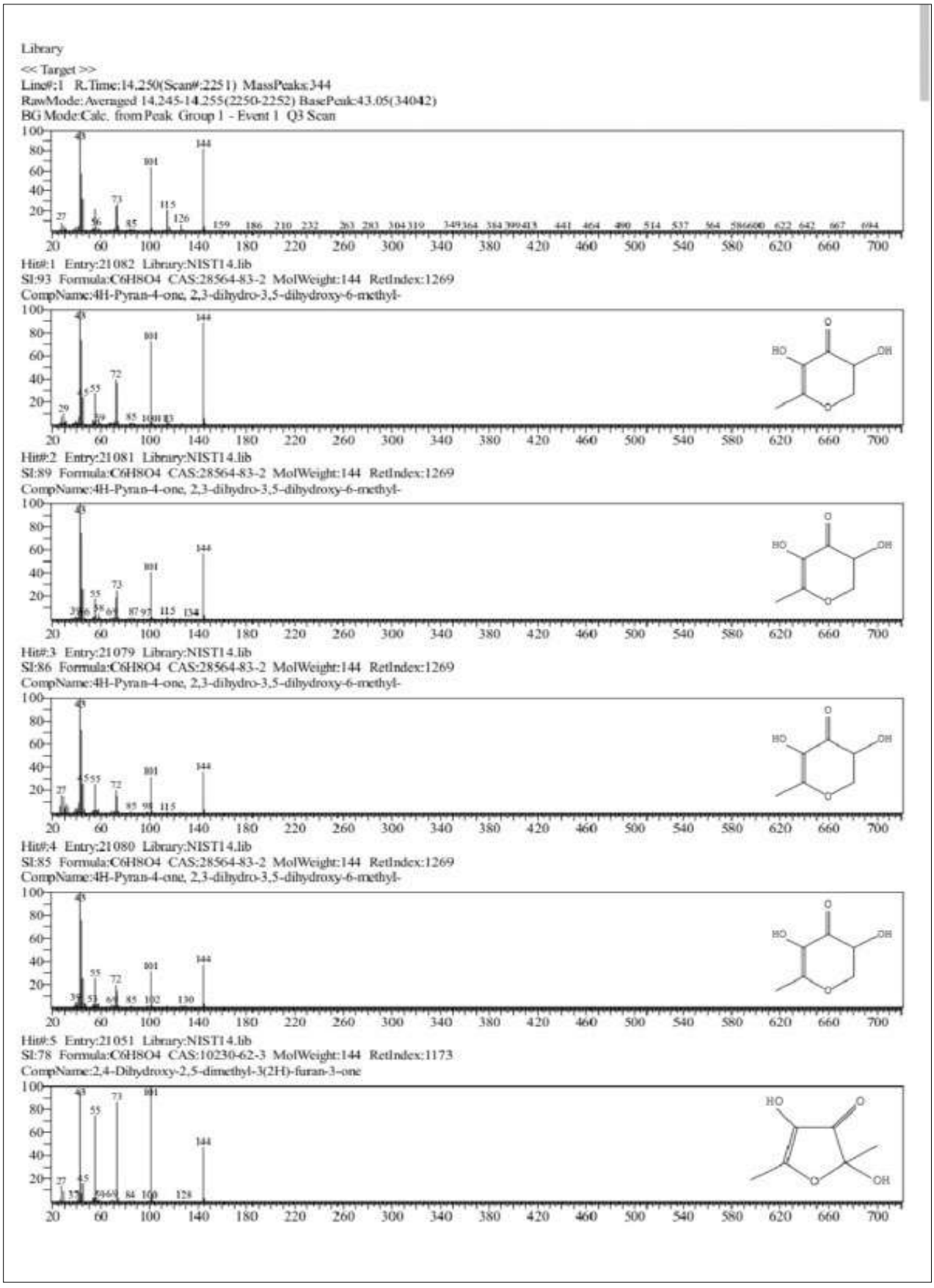
Figure 1. GC-MS Chromatogram of Methanolic Fruits extract of *Garuga pinnata* (Roxb.)

The results of GC-MS analysis of methanol extract revealed the presence of 08 Major compounds and 17 Minor compounds. These identified compounds with their retention time (RT), Molecular Formula, Molecular weight and Concentration (Peak area %) are Presented in Table.1 and Table 2. The GC-MS chromatogram of Major 08 Compounds with their chemical structures is depicted in Figure 1. The GC-MS Spectrum Confirmed the Presence of 08 Components with the Retention time; 14.248, 17.348, 23.877, 25.526, 29.543, 35.029, 35.788 and 39.615.

Retention Time (Min)	% Area of Peak	Compound Analyzed	Molecular Formula	Mol.Wt. (In grams)	CAS Number	Functional Group
14.248	2.40	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	28564-83-2	Hydroxy or Corbonyl
17.348	32.06	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	67-47-0	Alcohol
23.877	1.68	1,2,3-Benzenetriol	$C_6H_6O_3$	126	87-66-1	Hydroxy
25.526	1.21	Beta-D-Glucopyranose, 1,6-anhydro	$C_6H_{10}O_5$	162	498-07-7	Acid
29.543	61.37	Quinic acid	$C_7H_{12}O_6$	192	77-95-2	Acid
35.029	0.36	Butanoic acid,octyl ester	$C_{12}H_{24}O_2$	200	110-39-4	Acid
35.788	0.45	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	57-10-3	Acid
39.615	0.46	cis-13,16-Docasadienoic acid	$C_{22}H_{40}O_2$	336	7370-49-2	Acid

Table 1. Major bioactive Compounds identified in methanolic fruit extract of *G. pinnata*

In term of % peak area, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (2.40 %), 5-Hydroxymethylfurfural (32.06 %), 1,2,3-Benzenetriol (1.68 %), Beta-D-Glucopyranose, 1,6-anhydro (1.21%), Quinic acid (61.37 %), Butanoic acid,octyl ester (0.36%), n-Hexadecanoic acid (0.45%) and cis-13,16-Docasadienoic acid (0.46%) were found as Eight major Compounds in methanolic extract of *Garuga pinnata* Roxb.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SI:93 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269
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Hit#:2 Entry:21081 Library:NIST14.lib
SI:89 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269
CompName:4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
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SI:86 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269
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Hit#:4 Entry:21080 Library:NIST14.lib
SI:85 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269
CompName:4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
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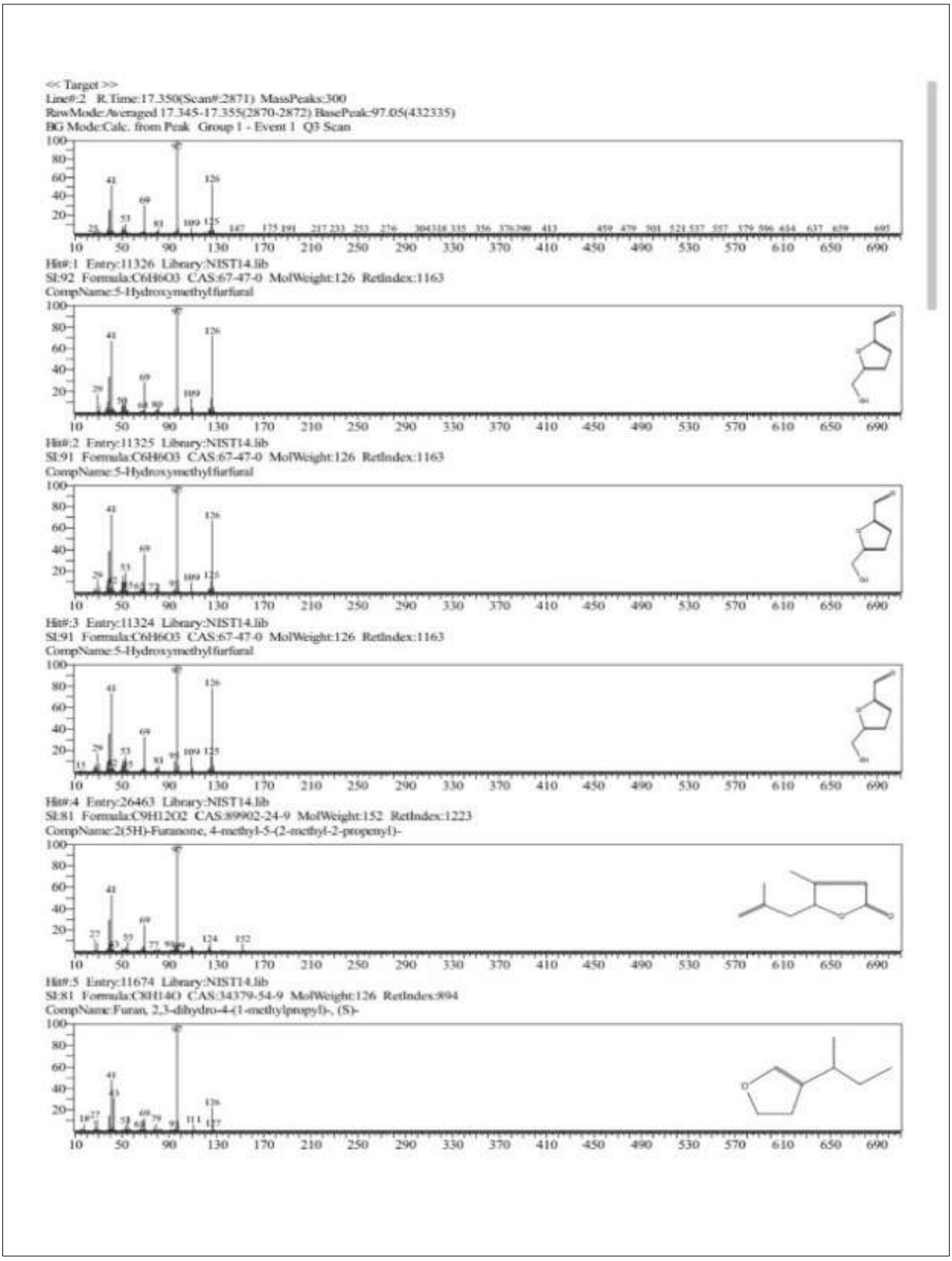
CompName:2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one

Figure 2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CompName:5-Hydroxymethylfurfural
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Hit#:5 Entry:11674 Library:NIST14.lib
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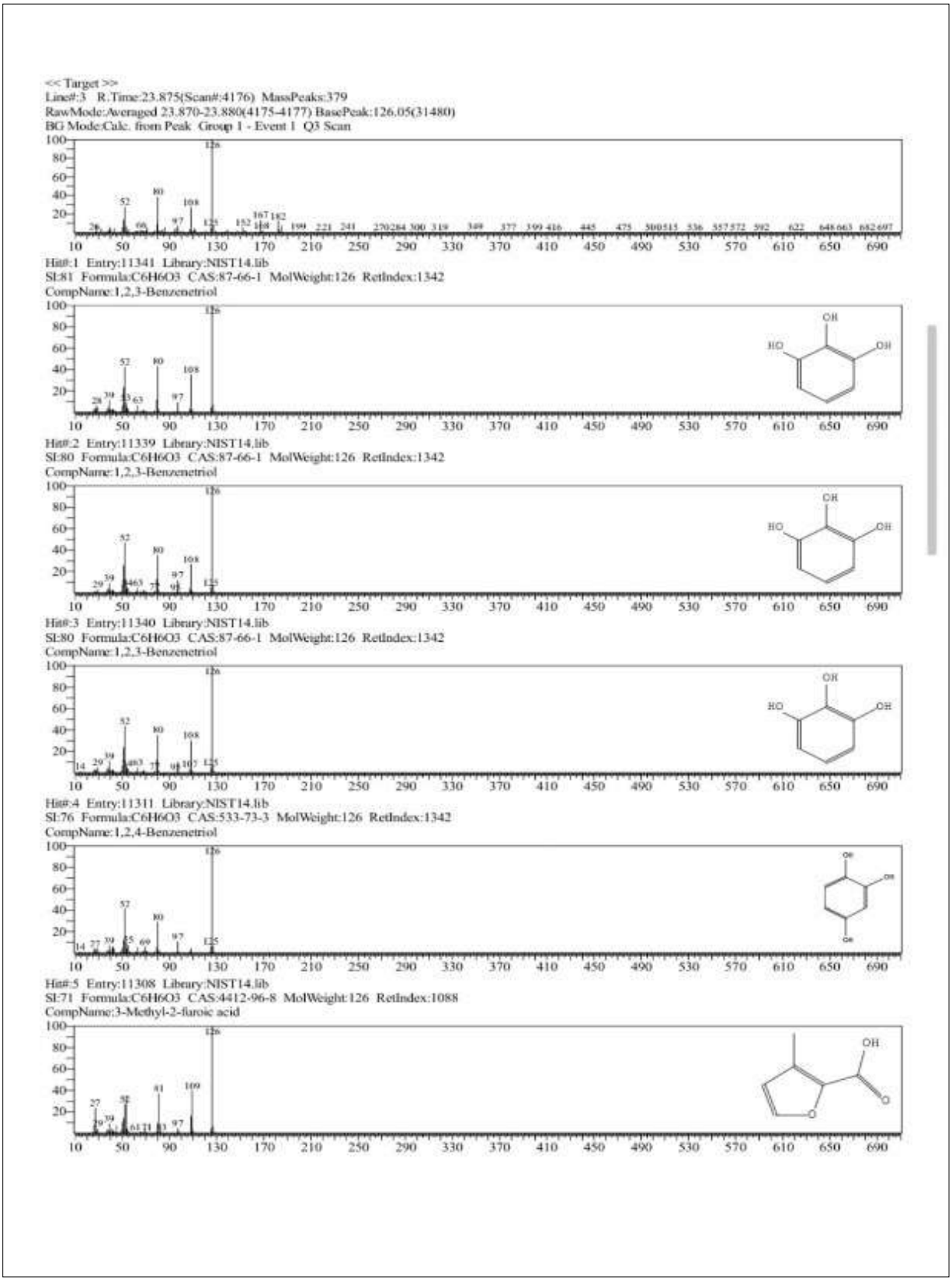
CompName:Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-

Figure 3.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CompName:1,2,4-Benzenetriol
Hit#:5 Entry:11308 Library:NIST14.lib
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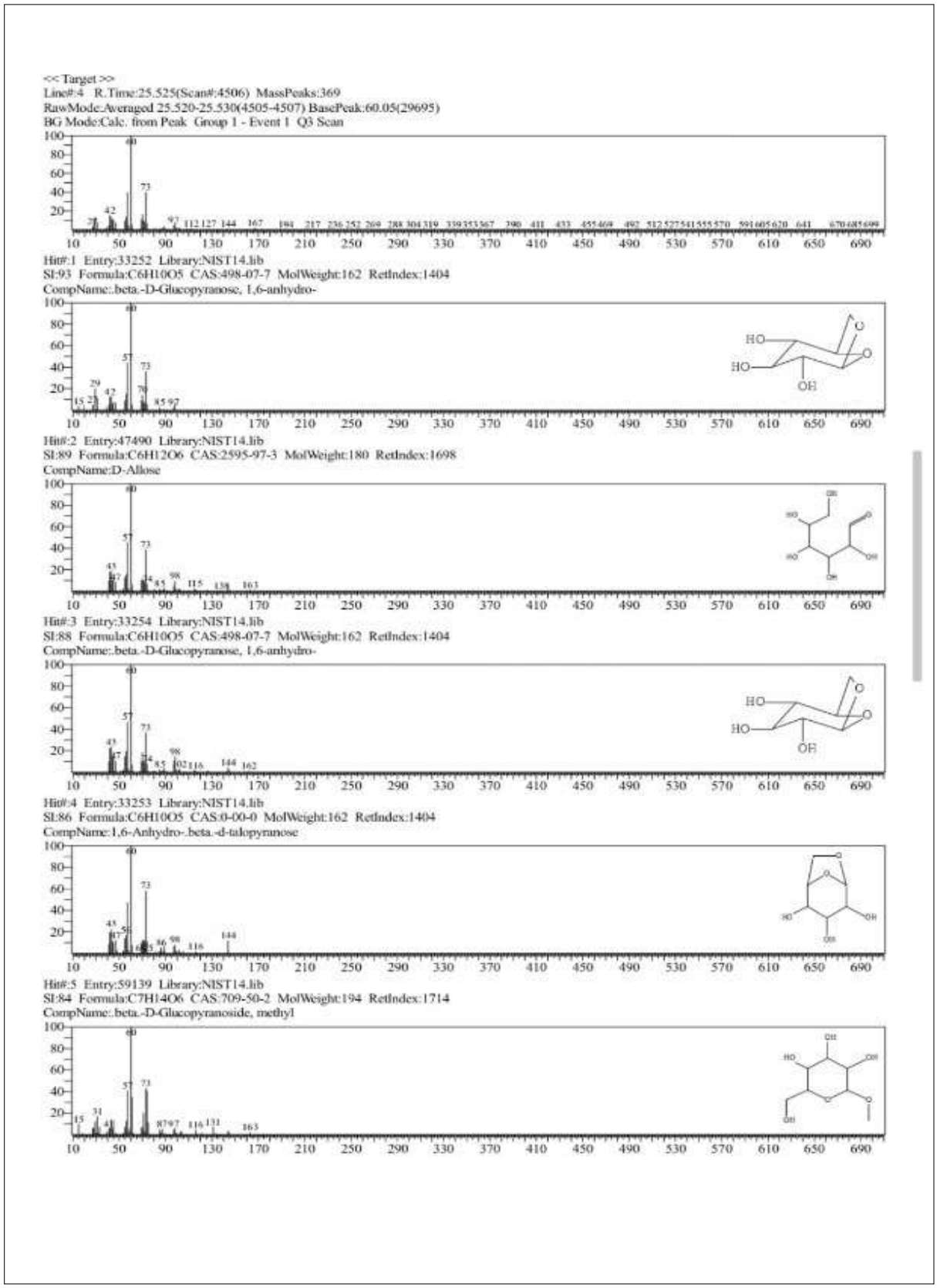
CompName:3-Methyl-2-furoic acid

Figure 4.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SI:89 Formula:C6H12O6 CAS:2595-97-3 MolWeight:180 RetIndex:1698
CompName:D-Allose
Hit#:3 Entry:33254 Library:NIST14.lib
SI:88 Formula:C6H10O5 CAS:498-07-7 MolWeight:162 RetIndex:1404
CompName:.beta.-D-Glucopyranose, 1,6-anhydro-
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SI:86 Formula:C6H10O5 CAS:0-00-0 MolWeight:162 RetIndex:1404
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Hit#:5 Entry:59139 Library:NIST14.lib
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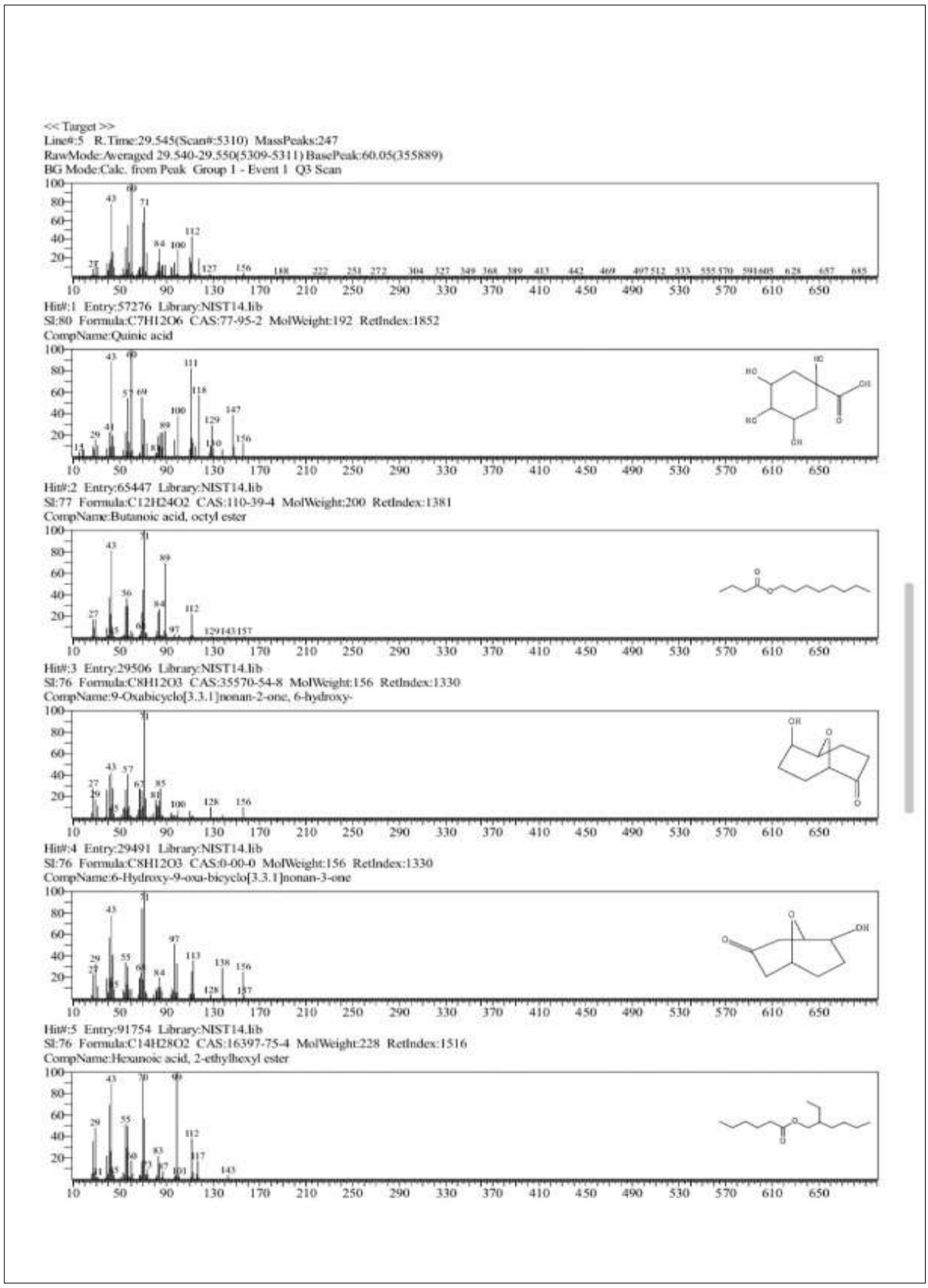
CompName:.beta.-D-Glucopyranoside, methyl

Figure 5.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SI:80 Formula:C7H12O6 CAS:77-95-2 MolWeight:192 RetIndex:1852
CompName:Quinic acid
Hit#:2 Entry:65447 Library:NIST14.lib
SI:77 Formula:C12H24O2 CAS:110-39-4 MolWeight:200 RetIndex:1381
CompName:Butanoic acid, octyl ester
Hit#:3 Entry:29506 Library:NIST14.lib
SI:76 Formula:C8H12O3 CAS:35570-54-8 MolWeight:156 RetIndex:1330
CompName:9-Oxabicyclo[3.3.1]nonan-2-one, 6-hydroxy-
Hit#:4 Entry:29491 Library:NIST14.lib
SI:76 Formula:C8H12O3 CAS:0-00-0 MolWeight:156 RetIndex:1330
CompName:6-Hydroxy-9-oxa-bicyclo[3.3.1]nonan-3-one
Hit#:5 Entry:91754 Library:NIST14.lib
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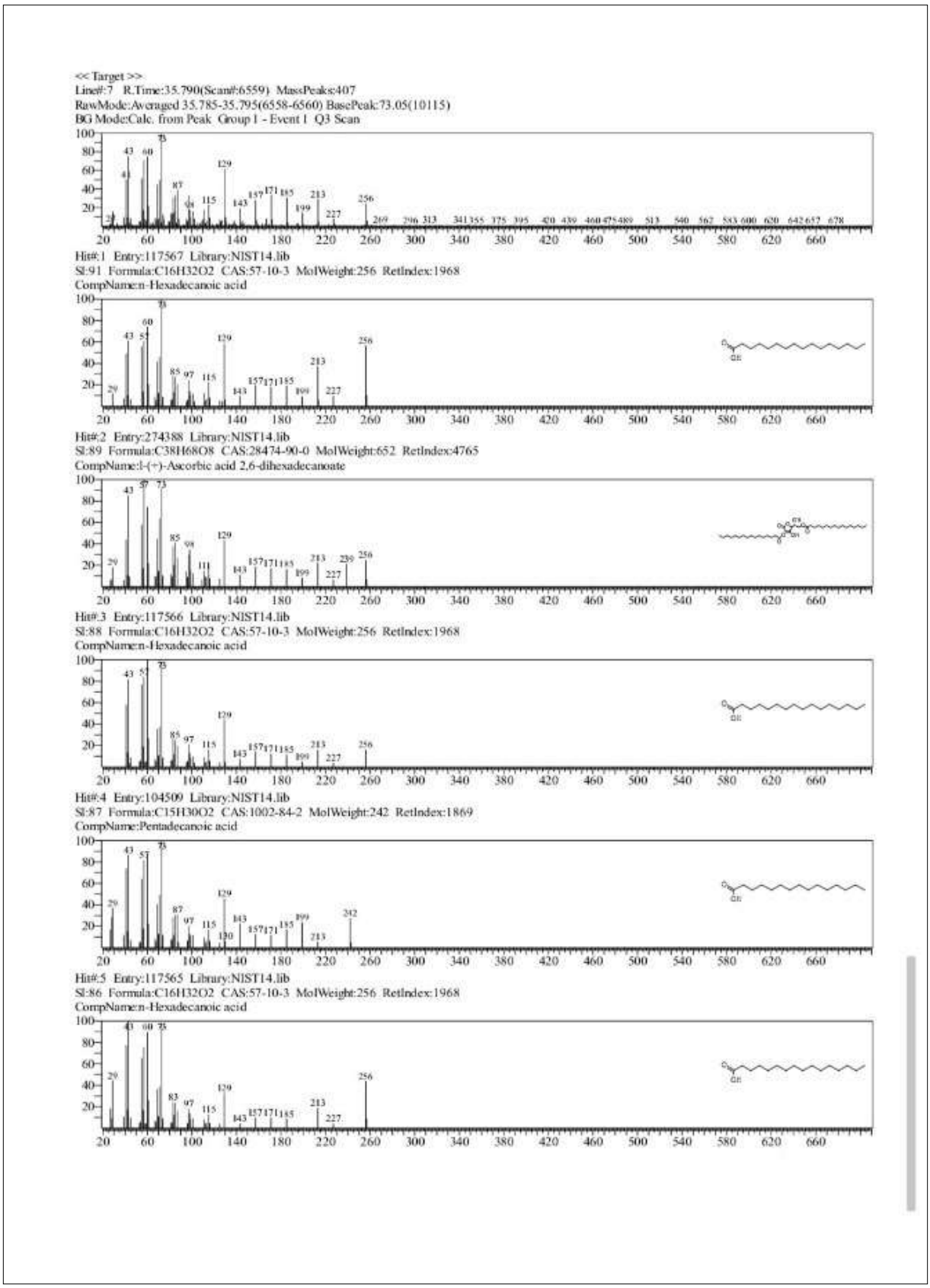
CompName:Hexanoic acid, 2-ethylhexyl ester

Figure 6.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CompName:n-Hexadecanoic acid
Hit#:2 Entry:274388 Library:NIST14.lib
SI:89 Formula:C38H68O8 CAS:28474-90-0 MolWeight:652 RetIndex:4765
CompName:l-(+)-Ascorbic acid 2,6-dihexadecanoate
Hit#:3 Entry:117566 Library:NIST14.lib
SI:88 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid
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SI:87 Formula:C15H30O2 CAS:1002-84-2 MolWeight:242 RetIndex:1869
CompName:Pentadecanoic acid
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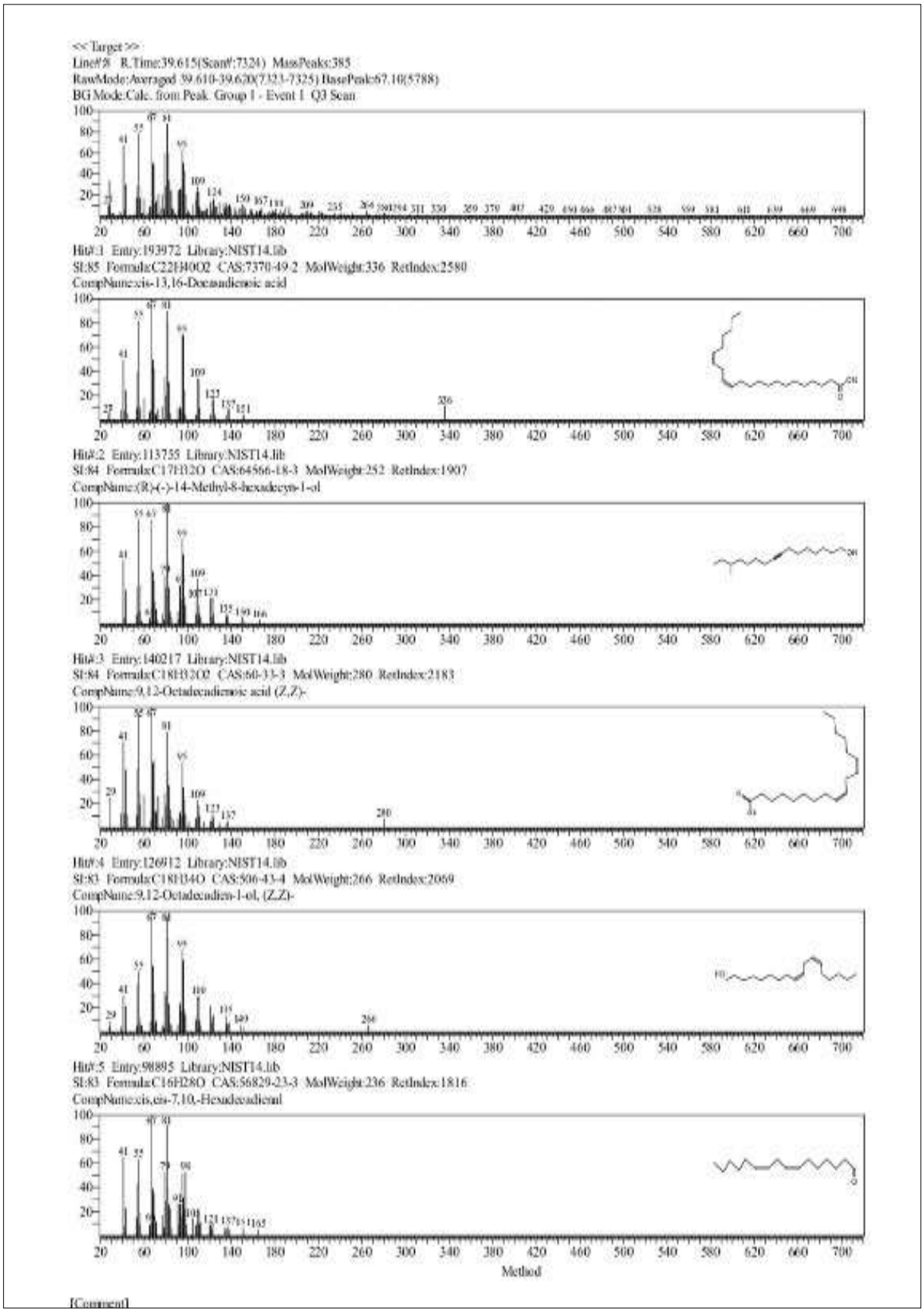
SI:86 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid

Figure 7.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CompName:cis-13,16-Docosadienoic acid
Hit#:2 Entry:113755 Library:NIST14.lib
SI:84 Formula:C17H32O CAS:64566-18-3 MolWeight:252 RetIndex:1907
CompName:(R)-(-)-14-Methyl-8-hexadecyn-1-ol
Hit#:3 Entry:140217 Library:NIST14.lib
SI:84 Formula:C18H32O2 CAS:60-33-3 MolWeight:280 RetIndex:2183
CompName:9,12-Octadecadienoic acid (Z,Z)-
Hit#:4 Entry:126912 Library:NIST14.lib
SI:83 Formula:C18H34O CAS:506-43-4 MolWeight:266 RetIndex:2059
CompName:9,12-Octadecadien-1-ol, (Z,Z)-
Hit#:5 Entry:98895 Library:NIST14.lib
SI:83 Formula:C16H28O CAS:56829-23-3 MolWeight:236 RetIndex:1816
CompName:cis,cis-7,10,-Hexadecadienal
Method
[Comment]

Figure 8

Figure 2.a) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl **b)** 2,4-Dihydroxy-2,5-dimethyl-3 (2H)-furan-3-one
Figure 3.a) 5-Hydroxymethylfurfural **b)** 2(5H)-Furanone,4-methyl-5-(2-methyl-2-Propenyl) **c)** Furan,2,3-dihydro-4-(1-methylpropyl)-, (S)
Figure 4.a) 1,2,3-Benzenetriol **b)** 3-Methyl-2-furoic acid
Figure 5.a) beta-D-Glucopyranose, 1,6-anhydro **b)** 1,6-Anhydro-beta-d-talopyranose **c)** D-Allose
Figure 6.a) Quinic acid **b)** Butanoic acid,octyl ester **c)** 9-oxabicyclo[3.3.1]nonan-2-one, 6-hydroxy **d)** Hexanoic acid, 2-ethylhexyl ester **e)** 6-Hydroxy-9-oxa-bicyclo [3.3.1]nonan-3-one
Figure 7. a) n-Hexadecanoic acid **b)** Pentadecanoic acid.
Figure 8. a) cis-13,16-Docasadienoic acid **b)** (R)-(-)-14-Methyl-8-hexadecyn-1-ol **c)** 9,12-Octadecadienoic acid (Z,Z) **d)** Cis, cis- 7,10,- Hexadecadienal

Table 2. Minor bioactive Compounds identified in Methanolic fruit extract of *G. pinnata*

Compound Analyzed	Molecular Formula	Mol.Wt. (In grams)	CAS No.	Functional Group
2,4-Dihydroxy-2,5-dimethyl-3 (2H)-furan-3-one	$C_6H_8O_4$	144	10230-62-3	ester
Furan,2,3-dihydro-4-(1-methylpropyl)-, (S)-	$C_8H_{14}O$	126	34379-54-9	Ketones
2(5H)-Furanone,4-methyl-5-(2-methyl-2-Propenyl)-	$C_9H_{12}O_2$	152	89902-24-9	Hydroxy
1,2,4-Benzenetriol	$C_6H_6O_3$	126	87-66-1	Hydroxy
3-Methyl-2-furoic acid	$C_6H_6O_3$	126	4412-96-8	Acid
1,6-Anhydro-beta-d-talopyranose	$C_6H_{10}O_5$	162	0-00-0	Acid
D-Allose	$C_6H_{12}O_6$	180	2595-97-3	aldehyde
Butanoic Acid,octyl ester	$C_{12}H_{24}O_2$	200	110-39-4	Acid
9-oxabicyclo[3.3.1]nonan-2-one, 6-hydroxy-	$C_8H_{12}O_3$	156	35570-54-8	Acid
Hexanoic acid, 2-ethylhexyl ester	$C_{14}H_{28}O_2$	228	16397-75-4	Acid
6-Hydroxy-9-oxa-bicyclo [3.3.1]nonan-3-one	$C_8H_{12}O_3$	156	0-00-0	Acid
1-(+)- Ascorbc acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	28474-90-0	Acid
Pentadecanoic acid	$C_{15}H_{30}O_2$	242	1002-84-2	Acid
(R)-(-)-14-Methyl-8—hexadecyn-1-ol	$C_{17}H_{32}O$	252	64566-18-3	Carbonyl
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	60-33-3	Acid
9,12-Octadecadien-1-ol,(Z,Z)-	$C_{18}H_{34}O$	266	506-43-4	aldehyde
Cis, cis- 7,10,- Hexadecadienal	$C_{16}H_{28}O$	236	56829-23-3	aldehyde

DISCUSSION

The identified major compounds possess some important biological potential for future drug development. The Quinic acid is predominant followed by Alcoholic compounds, 5-Hydroxymethylfurfural.The compound Quinic acid with highest % peak area (61.37%) has previously been reported for antioxidant, antidiabetic, anticancer, antimicrobial, antiviral activity. (Bhandari *et al*.2021). Hydroxymethylfurfural was reported to show anticancer activity against cancer cells by induction of cell apoptosis (Joel, O and Maharjan, R. 2021). D-Allos has been shown Anticancer, anti-tumor, anti-inflammatory, anti-oxidative, antihypertensive and immunosupressant activity. n-Hexadecanoic acid possesses some biological activity such as antioxidant, hypocholesterolemic, nematicide and pesticide activity. Pentadecanoic acid has antibacterial and anti-fungal activity. The above-mentioned isolated compounds from the methanol extract of *Garuga pinnata* fruits seem to

possess the reported biological activity (Murali *et al*.2013) and further study of these phytoconstituents may prove the medicinal importance in future.

CONCLUSION

In present study, many compounds were detected, which were rich in bioactive volatile compounds. Among these volatile compounds some are represents functional group of hydroxy, carbonyl, acid, keton, esters and aldehyde. The major compounds noticed were Quinic acid, Hexadecanoic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5-Hydroxymethylfurfural, 1,2,3-Benzenetriol, beta-D-Glucopyranose, 1,6-anhydro. Hence, the presence of some of the important bioactive volatile compounds will certainly prove the use of extract of selected wild edible plant parts for the preparation of soaps, shampoos, shaving cream, cosmetics, varnishes, detergent, grease etc. because of the presence of such volatile compounds in selected parts of wild edible plants, these may be highly demanded in the pharmaceutical and food industries (Mahadkar S.D and Jadhav V. 2018).

Garuga pinnata is a plant, traditionally used for the treatment of stomach problem,asthma, pulmonary infections, bone fractures, obesity, opacities in conjunctivitis. But till date, there are no reports on chromatographic analysis of methanolic extract of the plant. Here in, we first time report the presence of some important compounds in this plant isolated by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the wild edible plants and to identify the plants from their adulterants using isolated compounds as biomarker.

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