Preliminary Phytochemical Screening and GC-MS Analysis of the Bioactive Components of the Leaf of *Terminalia catappa*

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Abstract:- Terminalia catappa **is a family of combretaceae which constitutes of many bioactive components. One thousand grammes (1000g) of the leaf of *Terminalia catappa* was extracted with methanol using cold infusion techniques. Crude extract obtained, which was further partitioned with n-hexane, ethyl acetate, n-butanol and water to give n-hexane portion (1.638% $^{W}/_{W}$), dark green in colour, oily in texture, ethyl acetate portion (0.075% $^{W}/_{W}$), black in colour, gummy in texture, n-butanol portion (0.777% $^{W}/_{W}$), brown in colour, oily in texture and finally aqueous portion (2.997% $^{W}/_{W}$), dark brown in colour, powdered in texture. Preliminary phytochemical screening of the methanol crude extract and partitioned portions revealed the presence of some secondary metabolites such as cardiac glycoside, flavonoids, saponins, terpenoids, tannins and alkaloid. However, metabolites such as phlabotannins and glycosides were tested negative. The n-butanol portion was subjected to purification using column and thin layer chromatography methods. Fractions were obtained rerun, recombined and pooled based on their retardation factor (R_f). Pure isolate with retardation factor (R_f) 0.4 and melting point 286.00-287.00^{o}C which was subjected to Gas Chromatography Mass Spectrometry (GC-MS) and the result was compared using NIST library of compounds which are of therapeutic values.**

Keywords: ***Terminalia catappa*****, Phytochemicals, Bioactive Compounds**

I. INTRODUCTION

Human society used the medicinal plants to combat diseases, since the dawn of civilization (1). A number of alternative medicine systems exist in the eastern region of the Mediterranean (2). For thousands of years nature has been a source of medicinal agents and based on their uses in traditional medicine an impressive number of modern drugs have been isolated from natural sources (3). In fact, plants are rich source of different types of medicines because they produce a diverse range of bioactive molecules (4). In the pharmaceutical industries natural products play an important role in drug development programs, therefore, over 50% of all modern clinical drugs are of natural product origin (5). In the addition of the importance of synthetic medicinal chemistry, there is huge interest in herbal medicine; there has been a revival of interest in herbal medicines to control major diseases and to discover new molecular structures as lead compounds from the plant kingdom (6). Terminalia catappa is from the family of combretaceae has been reported to contains many compounds of therapeutic values such as hydrolyzable tannins punicalagin (major tannin), punicalin, terflavins A and B,tergallagin, tercatain, chebulagic acid, geraniin, granatin B, corilagin), flavanoids (isovitexin, vitexin, isoorientin, rutin) and triterpinoids (ursolic acid, 2α, 3β, 23-trihydroxyurs-12-en-28oic acid and asiatic acid) [7]. This research work is aimed to expose the bioactive compounds present in the leaf of *Terminalia catappa.*

II. EXPERIMENTAL SECTION

Sample collection and Identification

Fresh leaf of *Terminalia catappa* was collected at Maiduguri metropolis of Borno state and it was identified by a plant taxonomist. The plant leaf material was air-dried in the laboratory at room temperature and it was ground to fine powder using wooden mortar and pestle.

Sample Extraction

The ground leaf material (1,000g) was extracted with 85% methanol using maceration (cold infusion) method for 72 hours. The crude extract was concentrated under reduced temperature. The crude extract was then stored in a desiccators. The methanol extract of *Terminalia catappa* was partitioned with n-hexane until exhaustion. The aqueous fraction was partitioned with ethyl acetate and also partitioned exhaustively with n-butanol. The n-butanol portion and the aqueous portion were then evaporated using rotatory oven. The resulting masses were then weighed and kept in a desiccator for further analysis.

Preliminary phytochemical screening

The methanol crude extract together with the partition portions were subjected to phytochemical screening using standard procedures to identify the phytochemicals [8] [9] [10].

Isolation of Compounds and Spectroscopic Analysis

The n-butanol partitioned portion was subjected to column chromatography using the Gradient elution protocol through suitable mixed solvents system, ethyl acetate and n-butanol (11) silica gel of 60-120 mesh was used. It was further subjected and purified using preparative thin layer chromatography (T.L.C) with a suitable mixed solvent of dichloromethane (92.5%), methanol (5%) and acetic acid (2.5%). The isolated compound with a retardation factor (R_f) 0.4 and melting point 286.00-287.00^{o}C was subjected to Gas Chromatography-Mass Spectrometry to identify possible compounds using standard protocols.

III. RESULT AND DISCUSSION

Table 1: Phytochemical Screening of *Terminalia catappa* Methanol Crude extract, n-Hexane Portion, Ethyl acetate Portion, n-Butanol Portion and Aqueous Portion.

S/NO.	Test	MCE	NHP	EAP	NBP	AQP
1.	Carbohydrate	+	-	-	+	+
2.	Soluble starch	-	-	-	-	-
3.	Phlabotannins	-	-	-	-	-
4.	Glycosides	-	-	-	-	-
5.	Cardiac glycoside	+	-	-	+	+
6.	Flavonoid	+	-	-	+	+
7.	Terpenoid	+	+	+	-	+
8.	Saponins	+	-	-	-	+
9.	Alkaloid	+	-	-	+	-
10.	Tannins	+	-	-	+	+

Key: MCE = Methanol Crude Extract, NHP = n-Hexane Portion, EAP = Ethyl Acetate Portion, NBP= n-Butanol Portion, AQP = Aqueous Portion, (+) = Present and (-) = Absent.

The results of phytochemical screening of *Terminalia catappa* leaf extract indicate that the plant contains many secondary metabolites. The methanol crude extract showed the presence of tannins, cardiac glycoside, flavonoid, terpenoid saponins and alkaloid while glycoside, phlabotannins and were not found in the extract. The *n*-hexane and ethyl acetate partitioned portion showed the presence of terpenoid only, while tannins, cardiac glycoside, flavonoid, saponins, alkaloid, glycoside, phlabotannins and soluble starch were not found. The *n*-butanol partitioned portion was found to contain carbohydrate, tannins, cardiac glycoside flavonoid and alkaloid. However, metabolites such as phlabotannins, terpenoid, saponins, soluble starch and glycosides were not found. Aqueous partitioned portion was also found to contain carbohydrate, tannins, cardiac glycoside, terpenoid, saponins and flavonoid. However, metabolites such as soluble starch, alkaloid, phlabotannins and glycosides were not found. The presence of secondary metabolites in the *Terminalia catappa* such as tannins, cardiac glycoside, flavonoid, saponnins and Phenolic compound, indicates or implicate the medicinal value of it

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Mr. Umar Dan' Azumi

Chromatogram

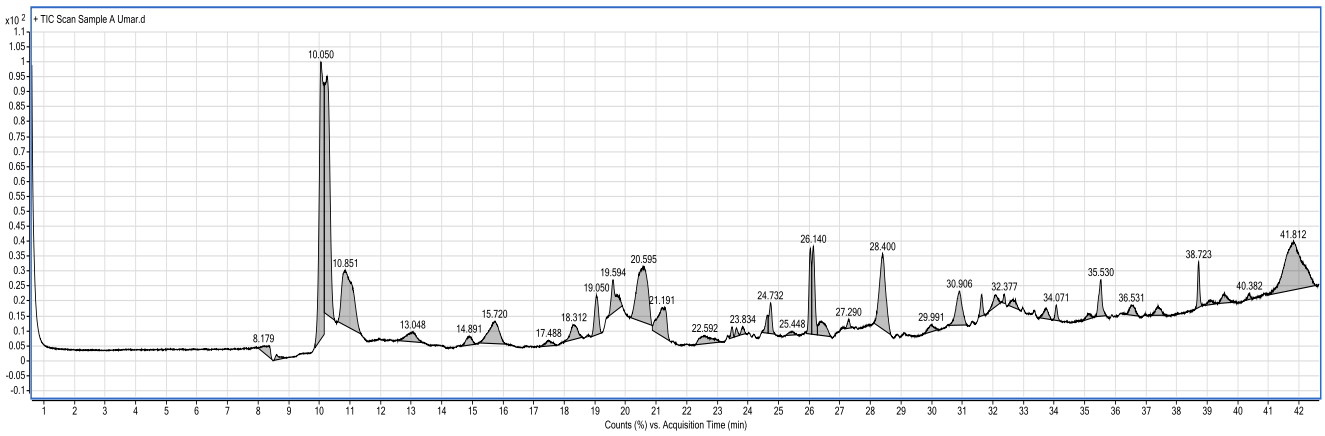
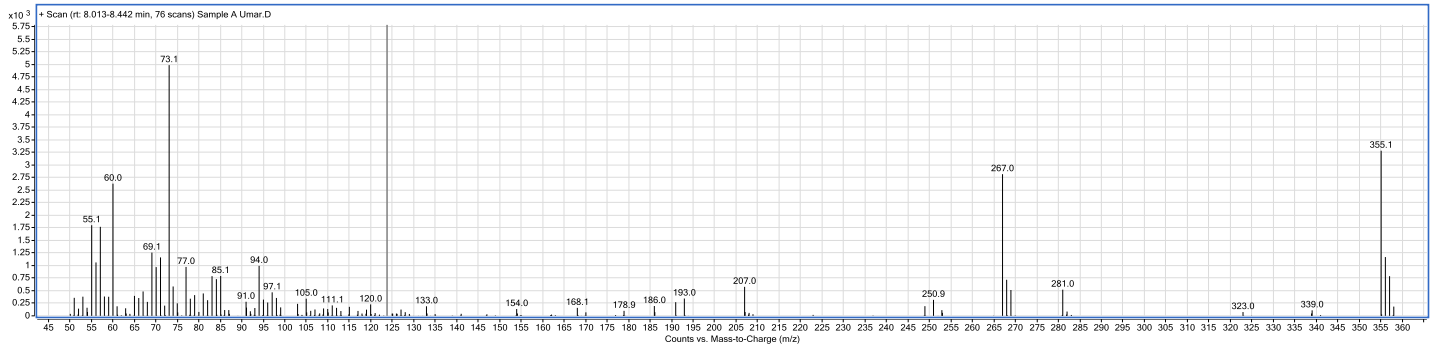
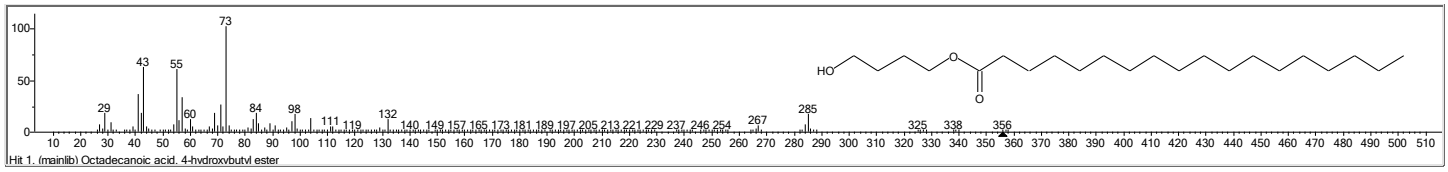


Fig. 1.0: Chromatogram

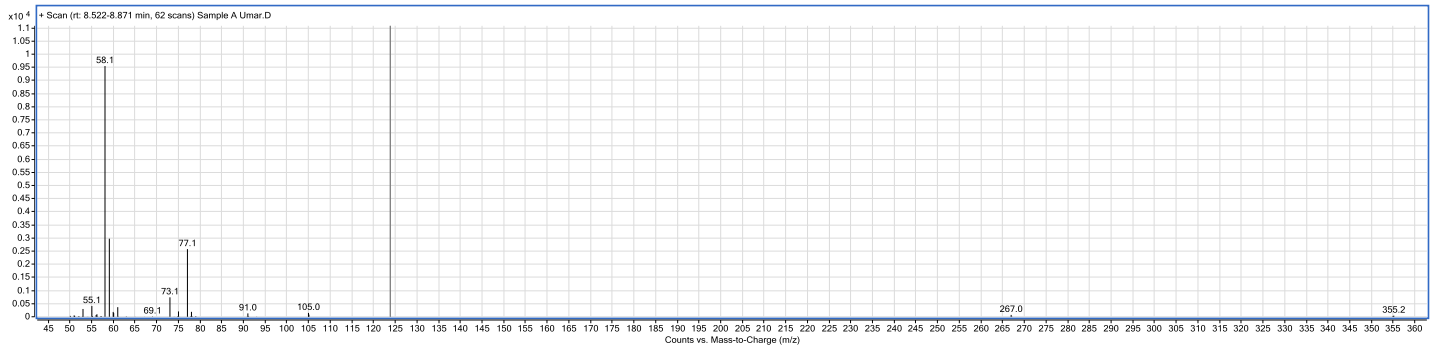
The chromatogram revealed the presence of forty two (42) compounds, but nineteen (19) compounds were having the comparison from the National Institute Standard and Technology (NIST) library such as Octadecanoic acid, 4-hydroxybutyl ester. It is important for fetal growth and development particularly for the central nervous system, affecting visual activity as well as cognitive function (12) and Phen-1-4 diol, 2,3-dimethyl-5- trifloromethyl which can be used as an antioxidant, antithrombotic and anti-tuberculosis [13, 14, 15], other peaks could not be identified from the library.



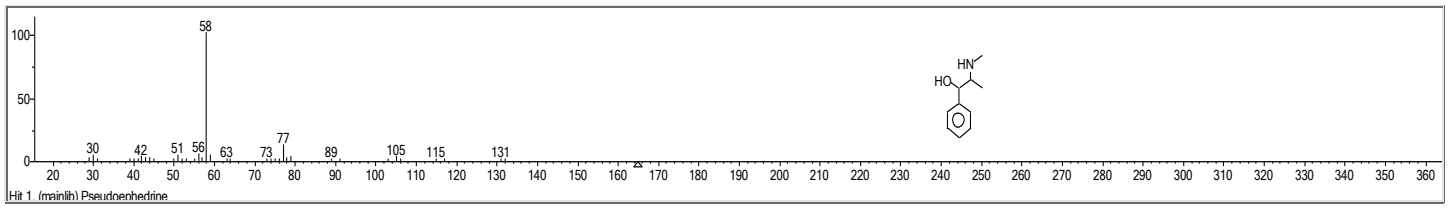
peak 1, rt: 8.179, base peak73.1, Mz 355.1



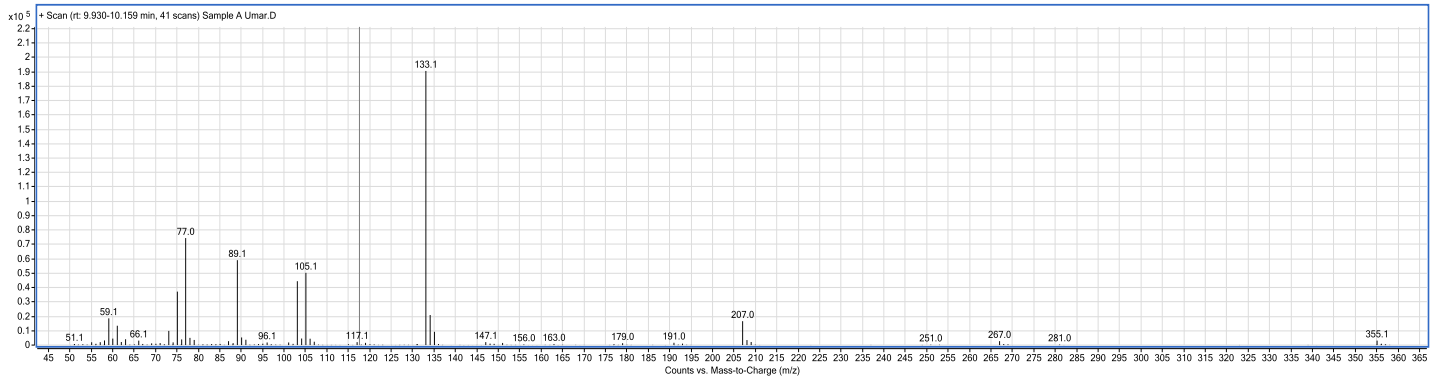
peak 1, mass spectrum comparison



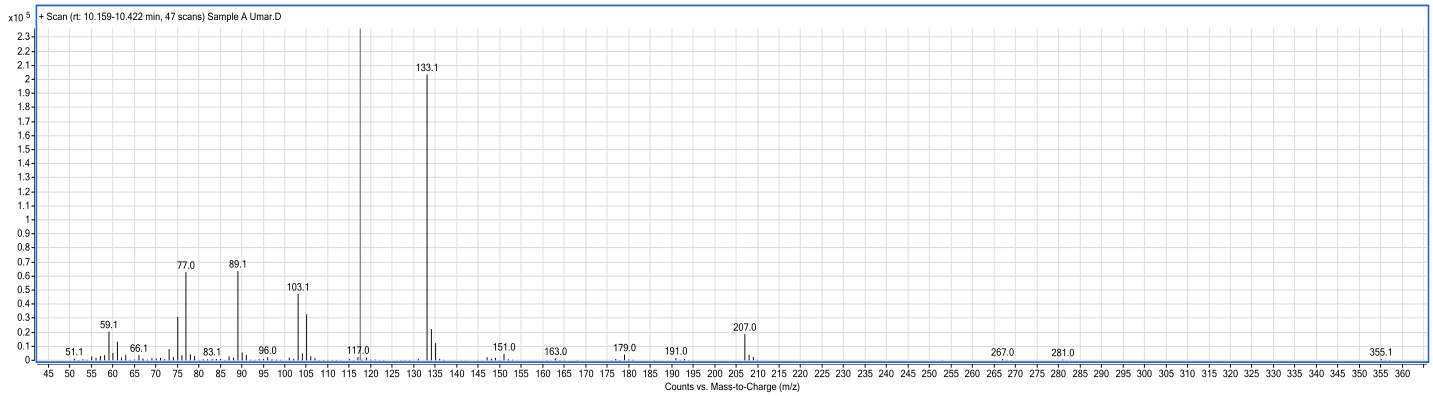
peak 2, rt: 8.608, base peak58.1 Mz 355.2



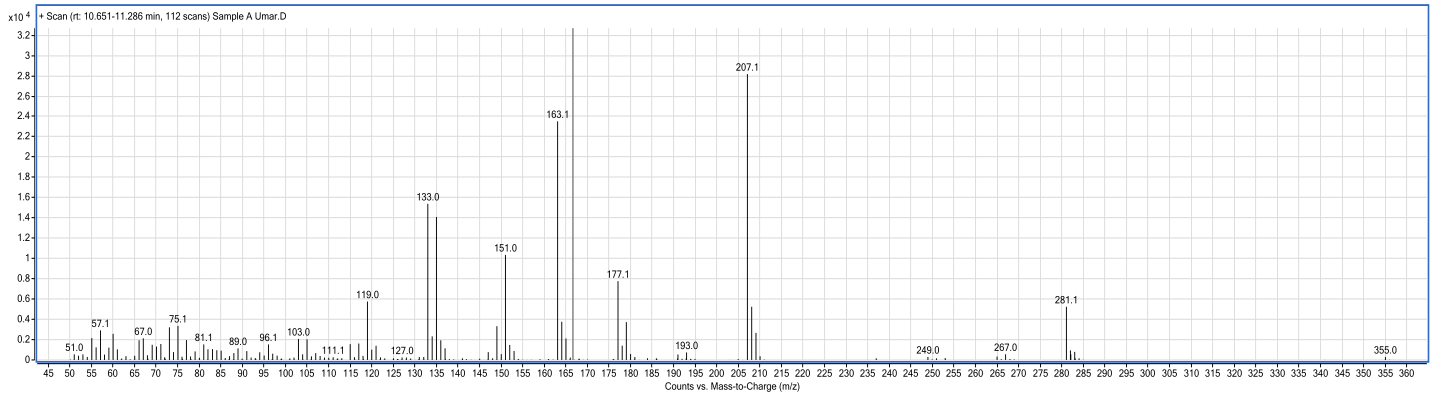
Peak 2, Ms Comparison



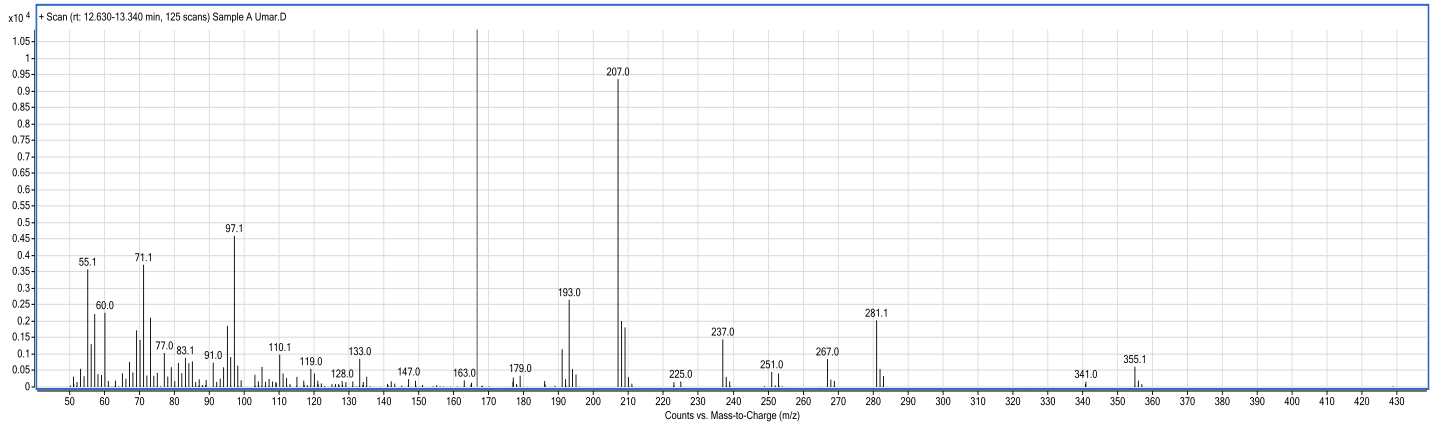
peak 3, rt: 10.050mins, base peak 133.1 Mz 355.1



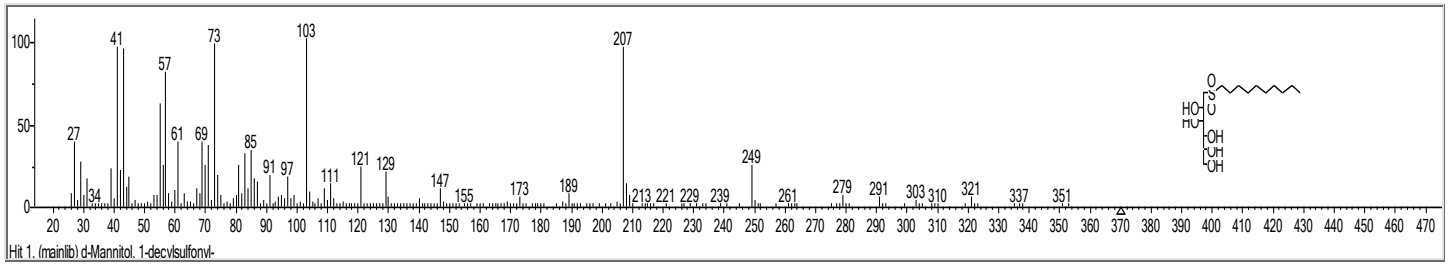
peak 4, rt: 10.250, base peak Mz



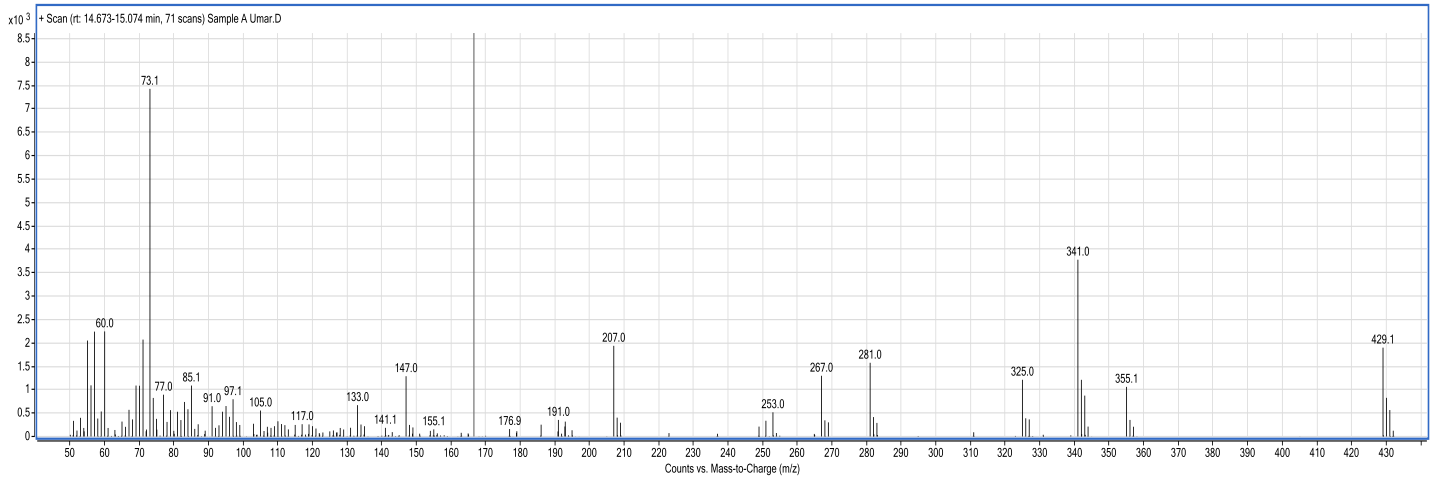
peak 5, rt: 10.851, base peak 207.1 Mz 355.0



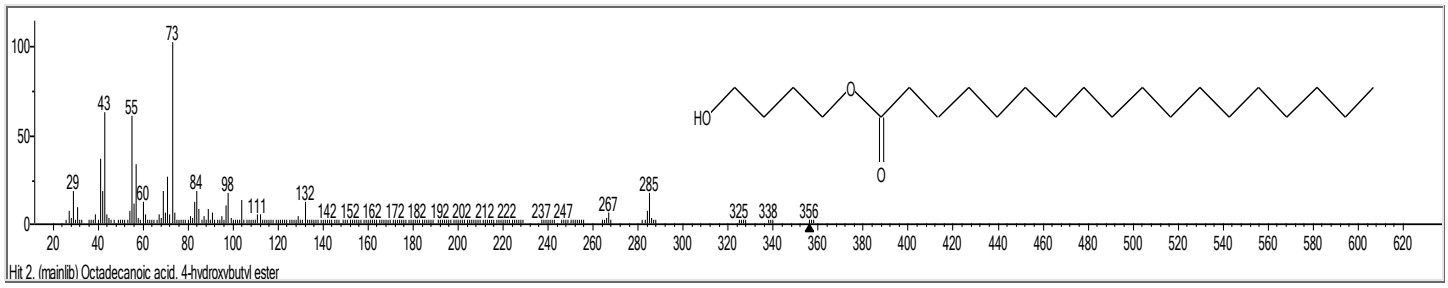
peak 6, rt: 13.048, base peak 207.1 Mz 355.1



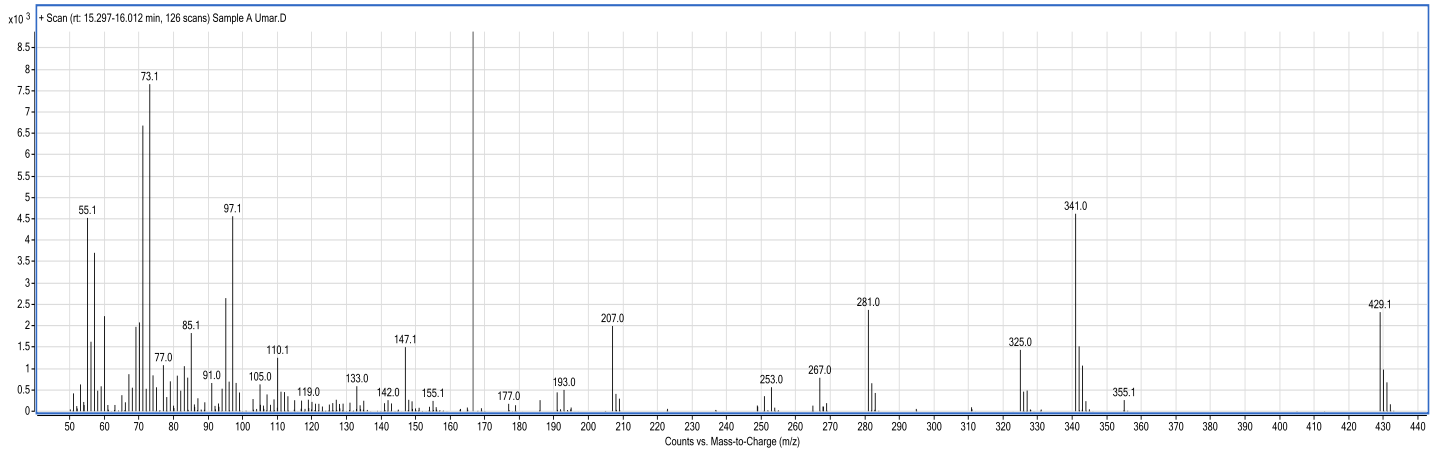
Peak 6 Ms comparison



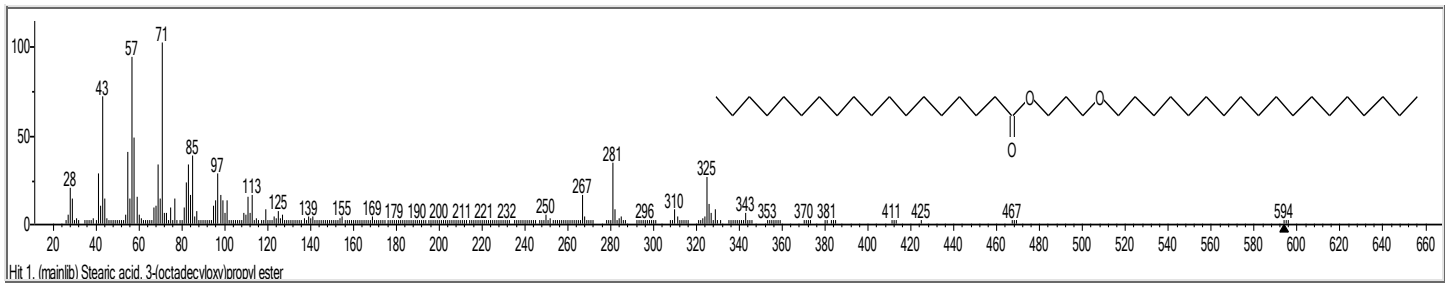
peak 7, rt: 14.891, base peak 73.1 Mz 429.1

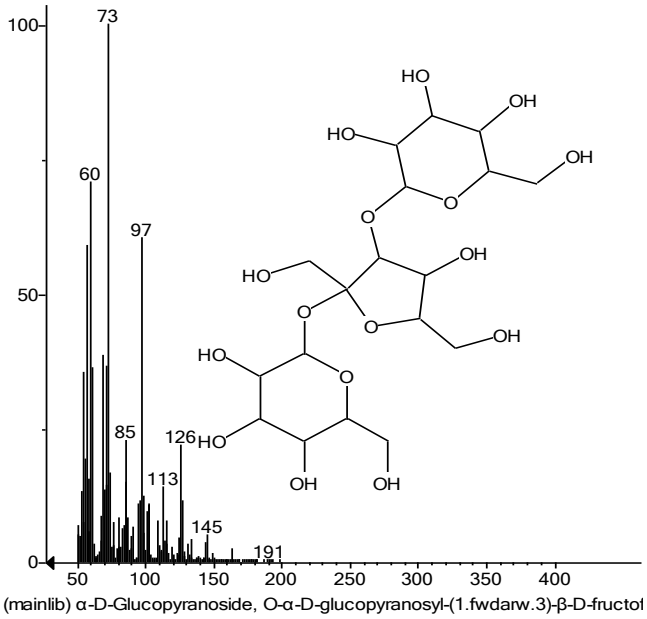


peak7 Ms comparison

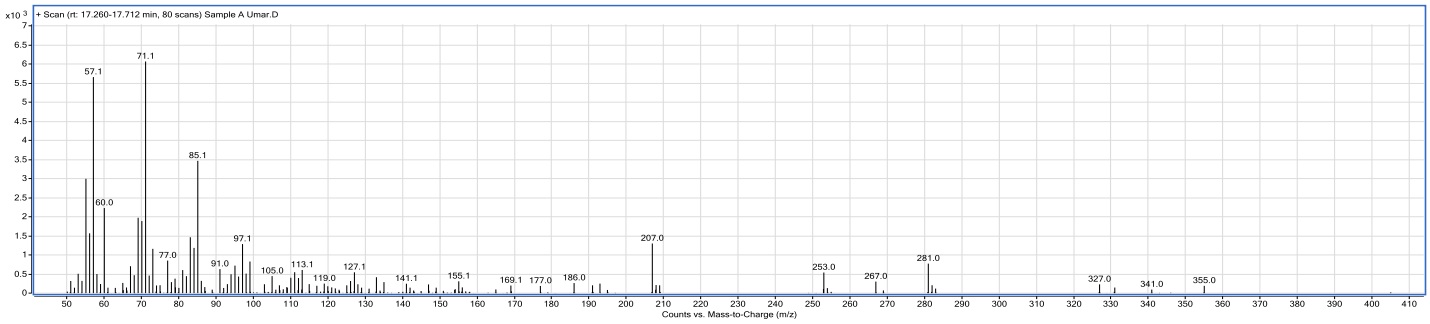


peak 8, rt: 15.720, base peak 73.1 Mz 429.1

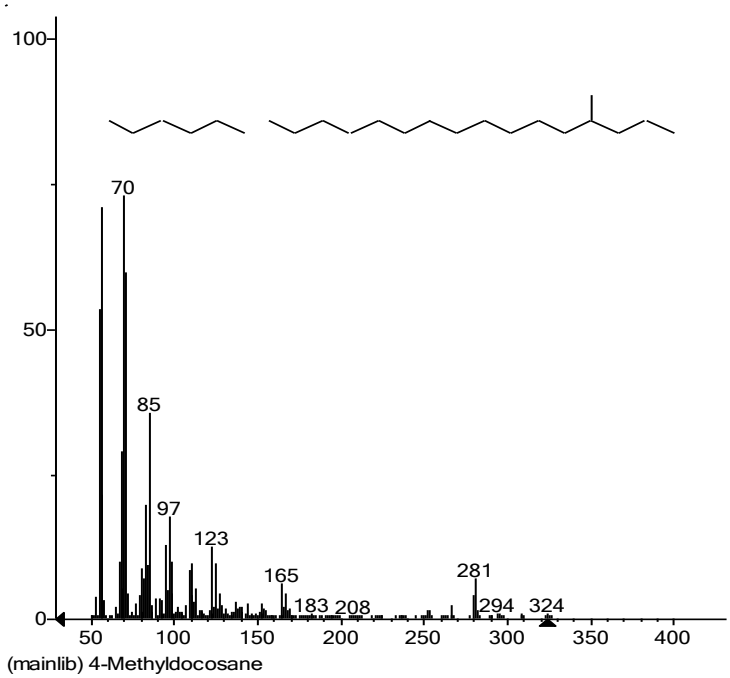




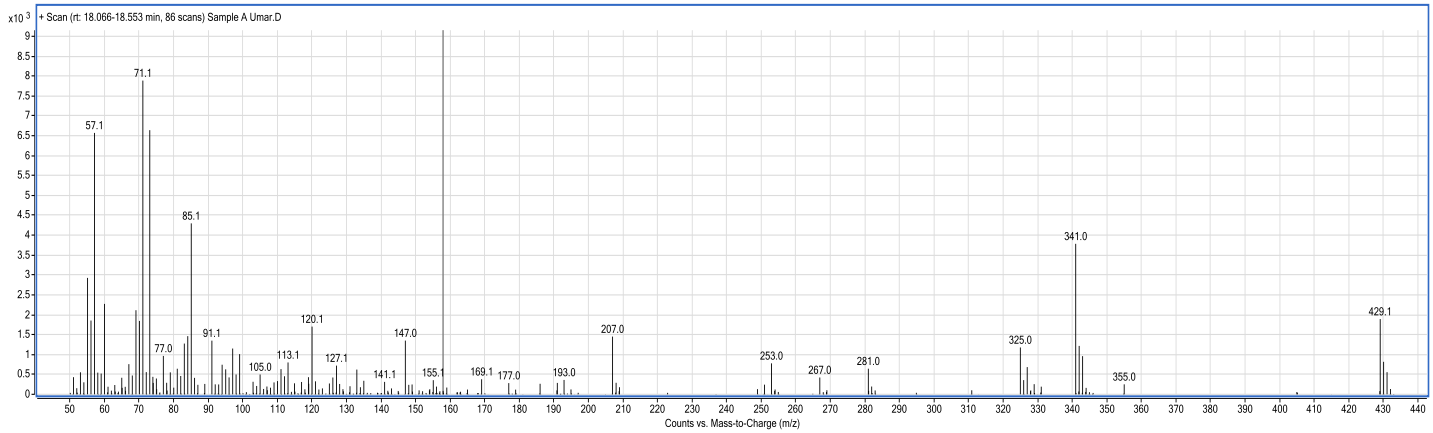
peak 8 Ms comparison



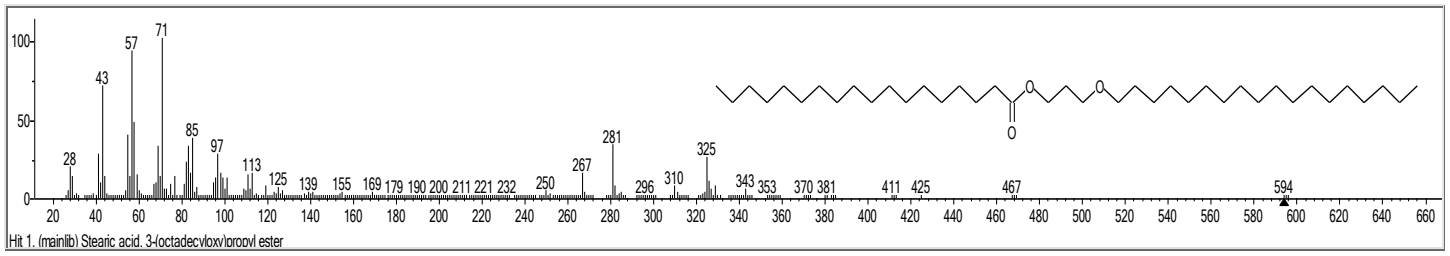
peak 9, rt: 17.488, base peak 71.1 Mz 355.0

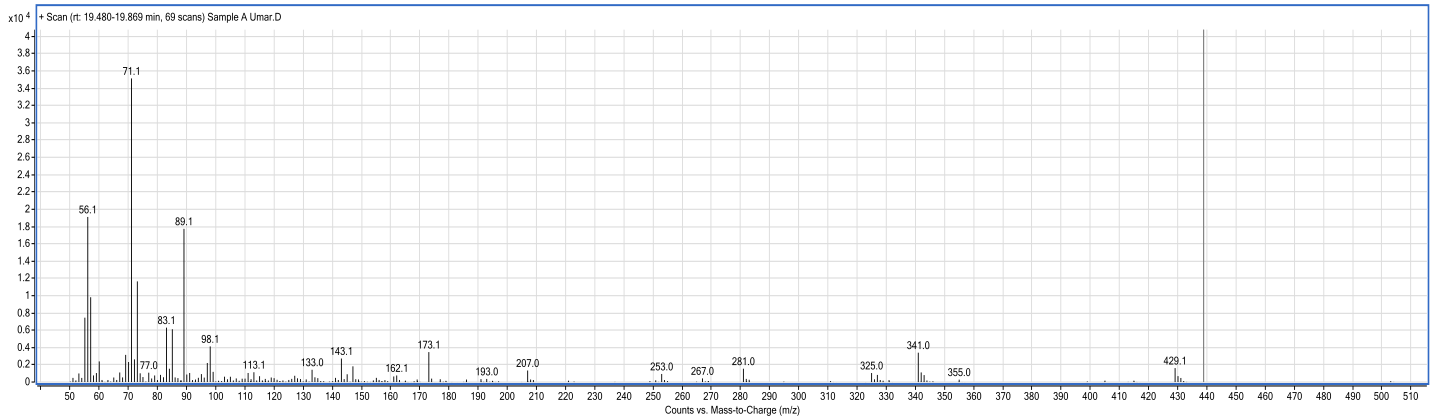


peak 10, rt: 18.312, base peak Mz

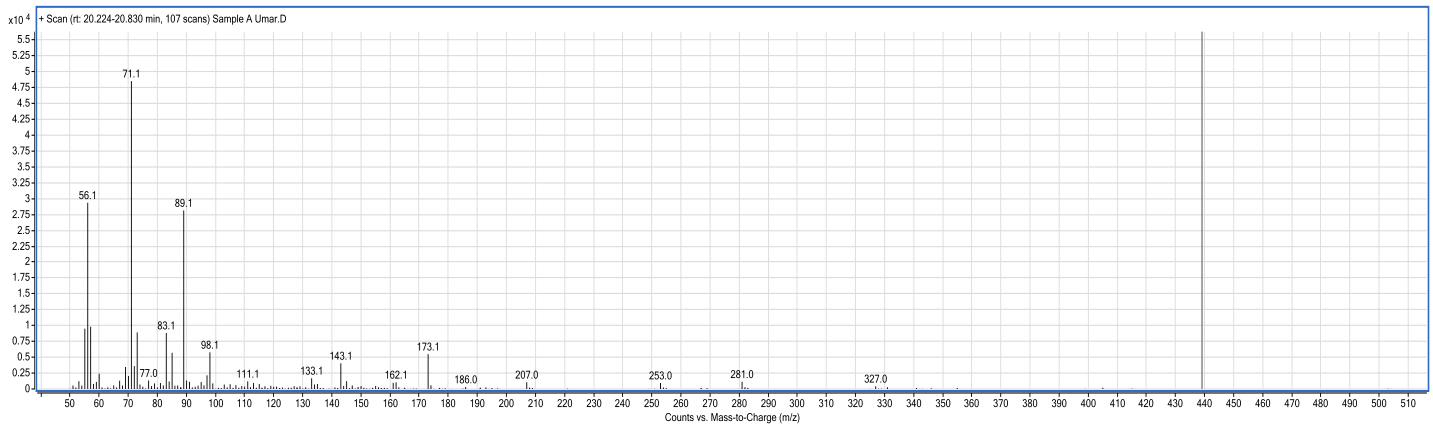


peak 11, rt: 19.050, base peak 71.1 Mz 429.1

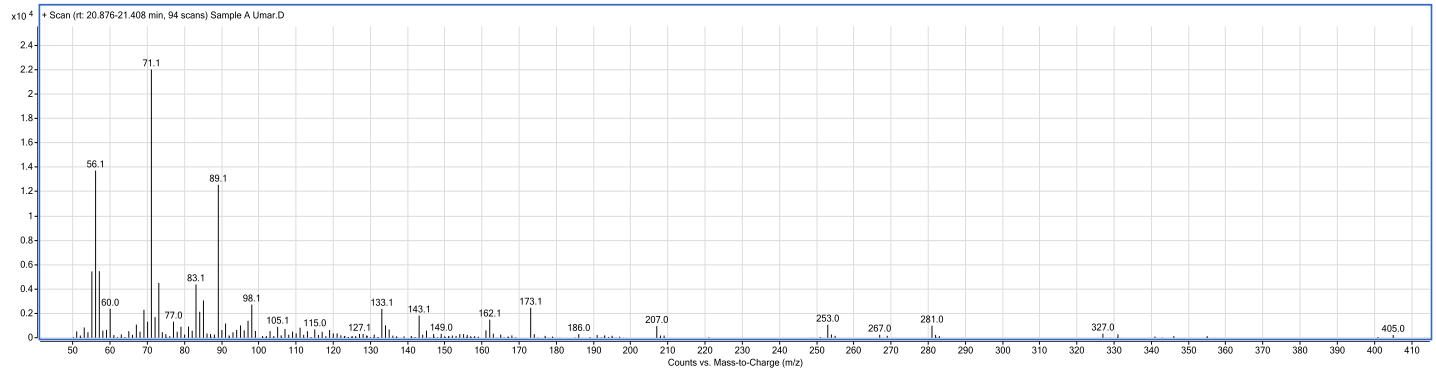




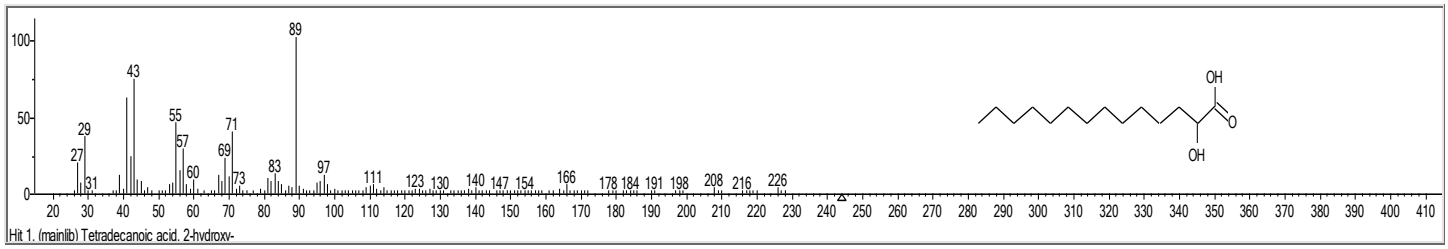
peak 12, rt: 19.594, base peak 71.1 Mz 429.1

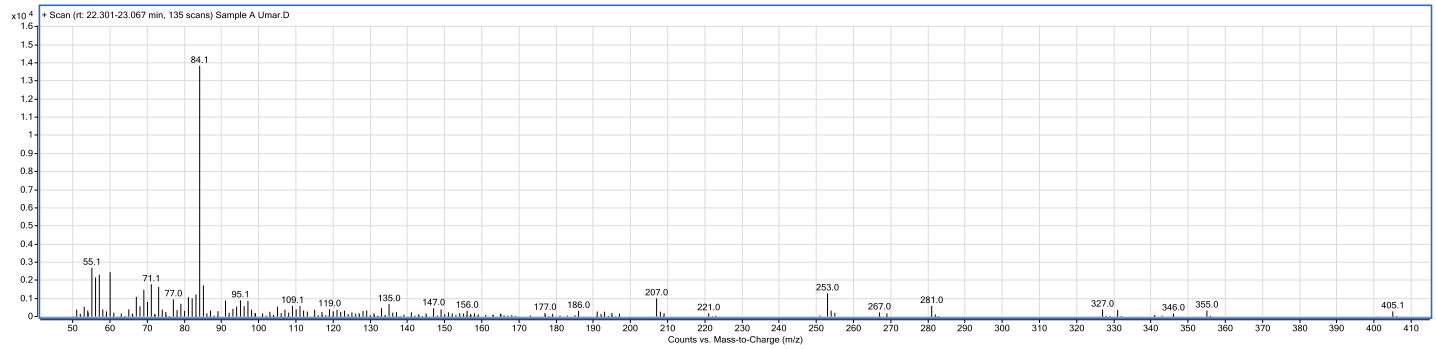


peak 13, rt: 20.595, base peak 71.1 Mz 327.1

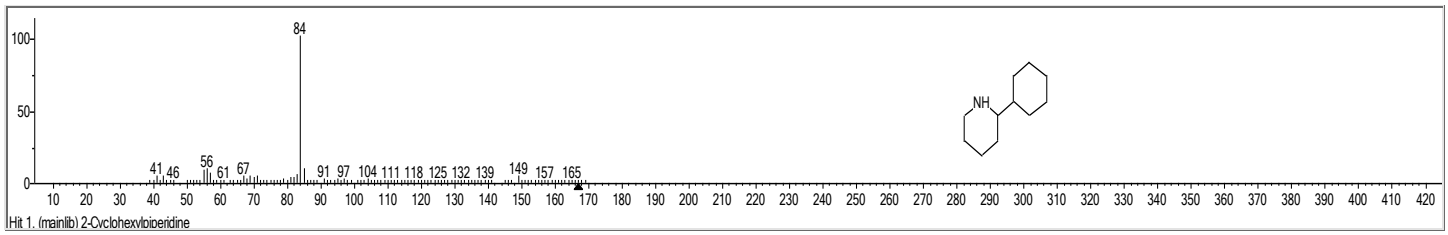


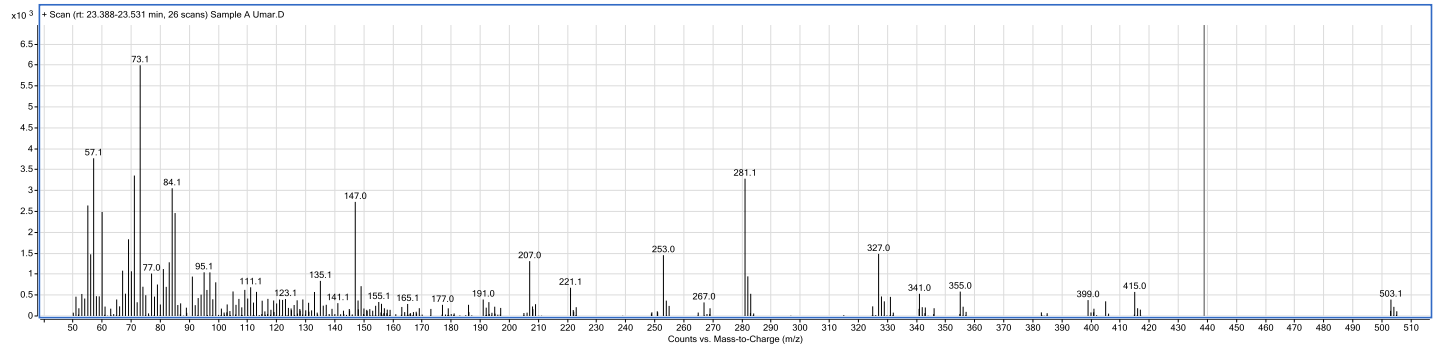
peak 14, rt: 21.190, base peak 71.1 Mz 405.1



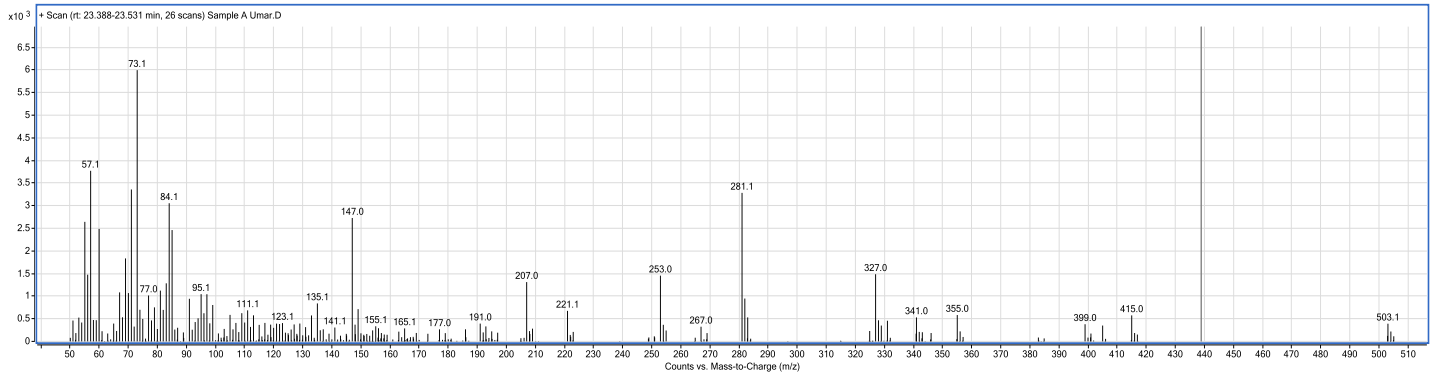


peak 15, rt: 22.592, base peak 84.1 Mz 405.1

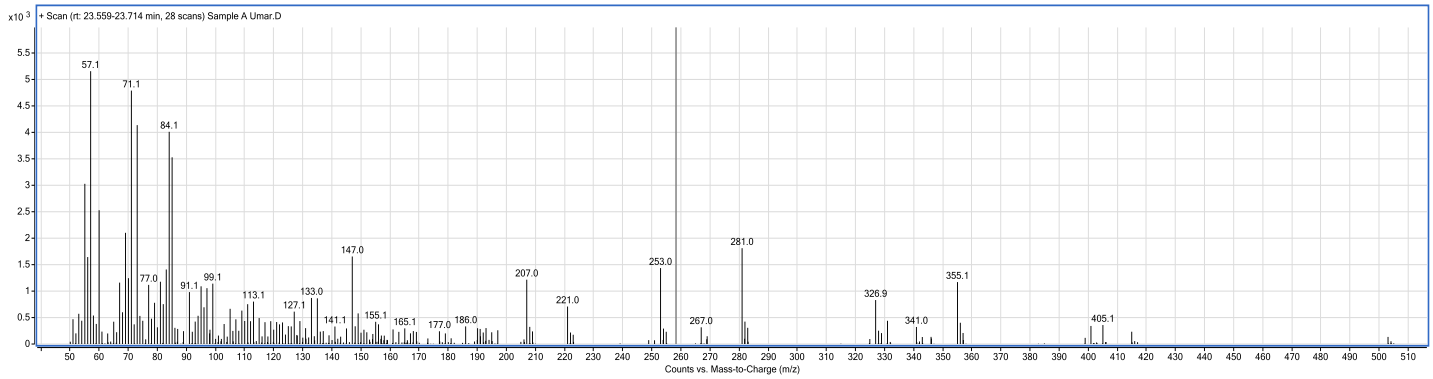




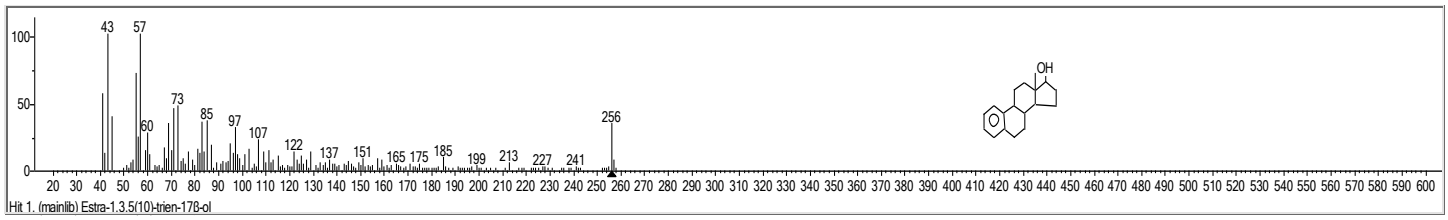
peak 16, rt: 23.479, base peak 73.1 Mz 503.1

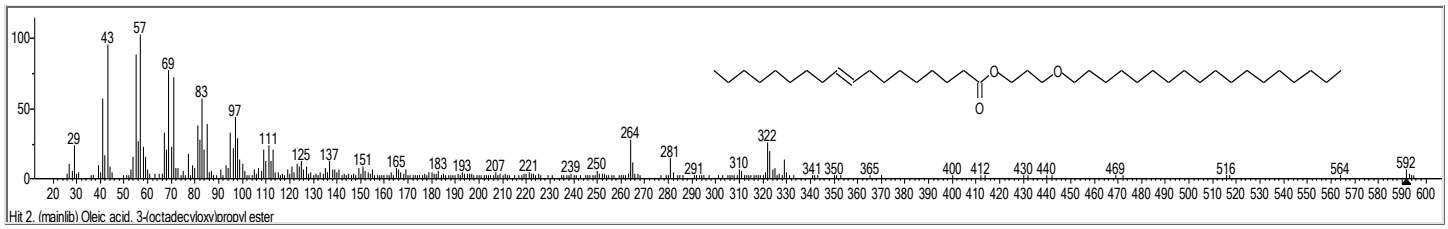


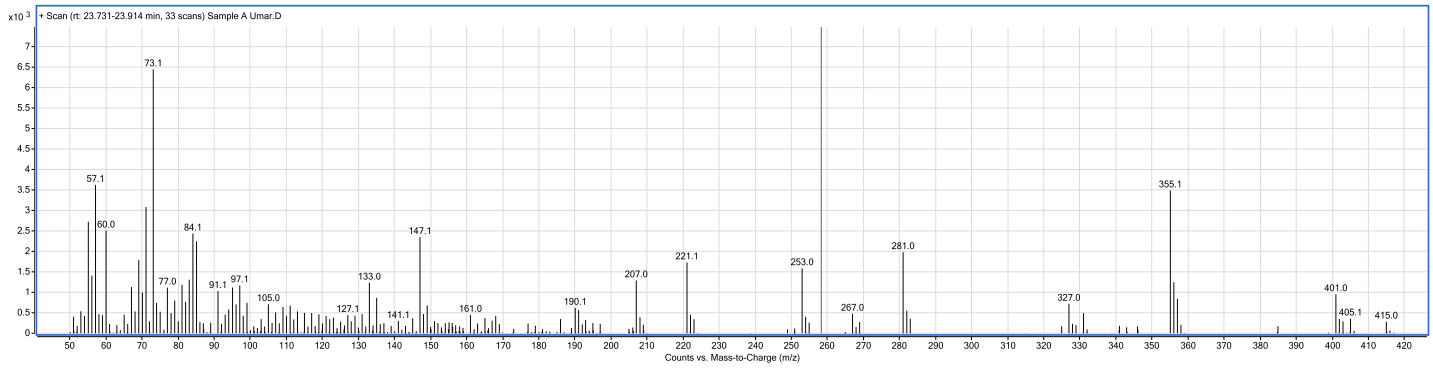
peak 17, rt: 23.622, base peak 73.1 Mz503.1



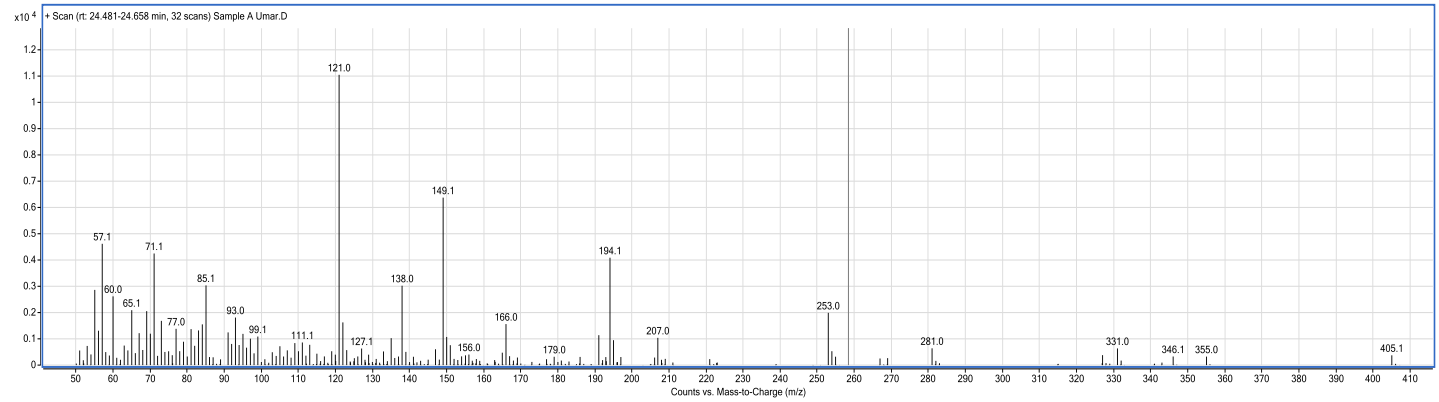
peak 18, rt: 23.834, base peak 57.1 Mz 405.1



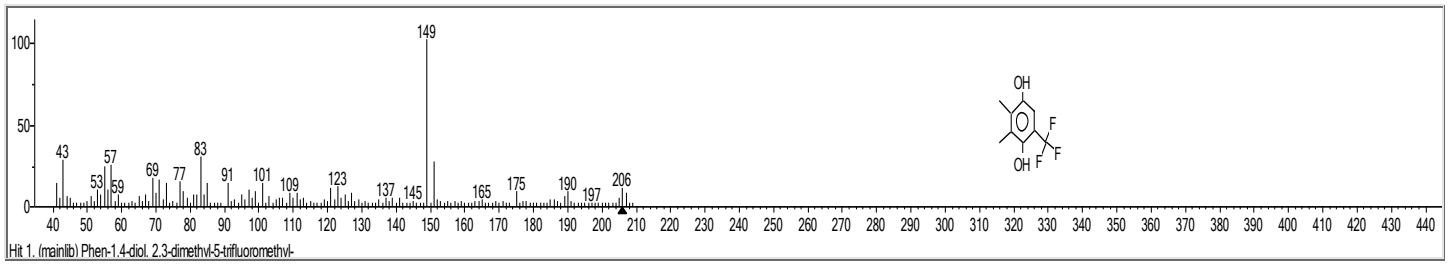


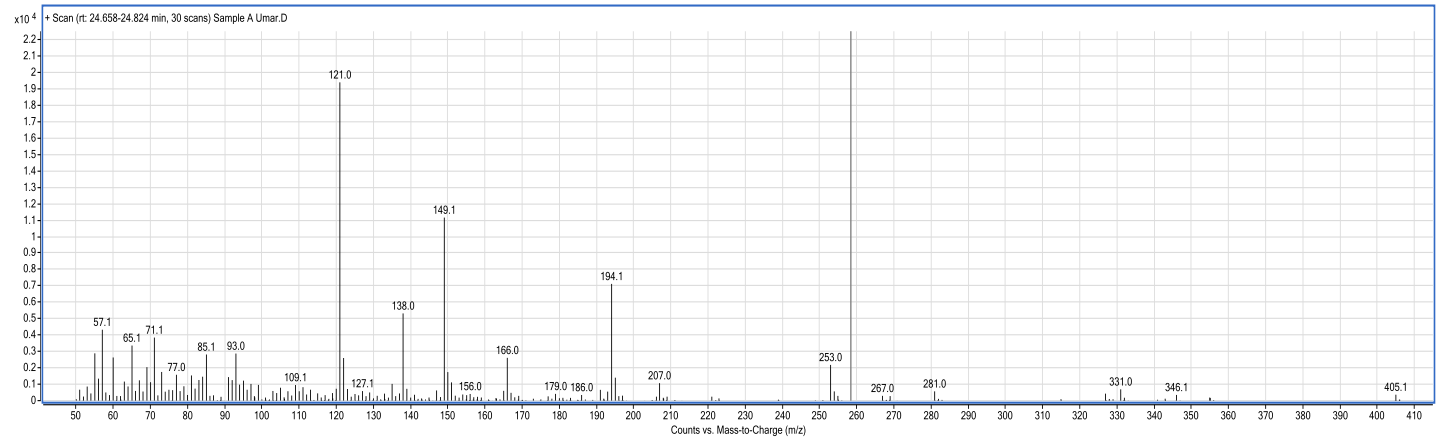


peak 19, rt: 24.629, base peak 73.1 Mz 415.0

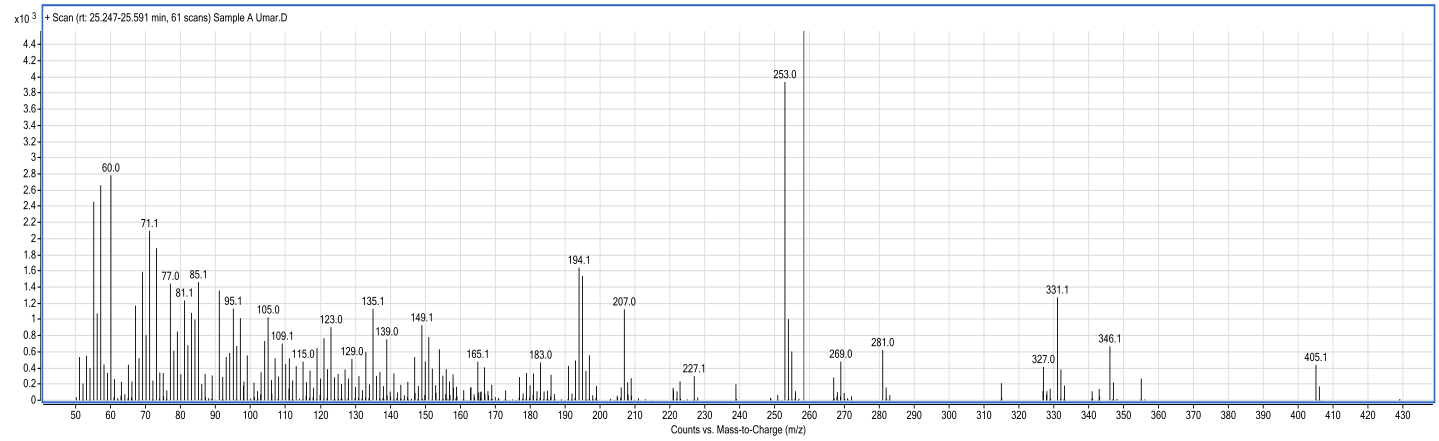


peak 20, rt: 24.732, base peak 121.0 Mz 405.1

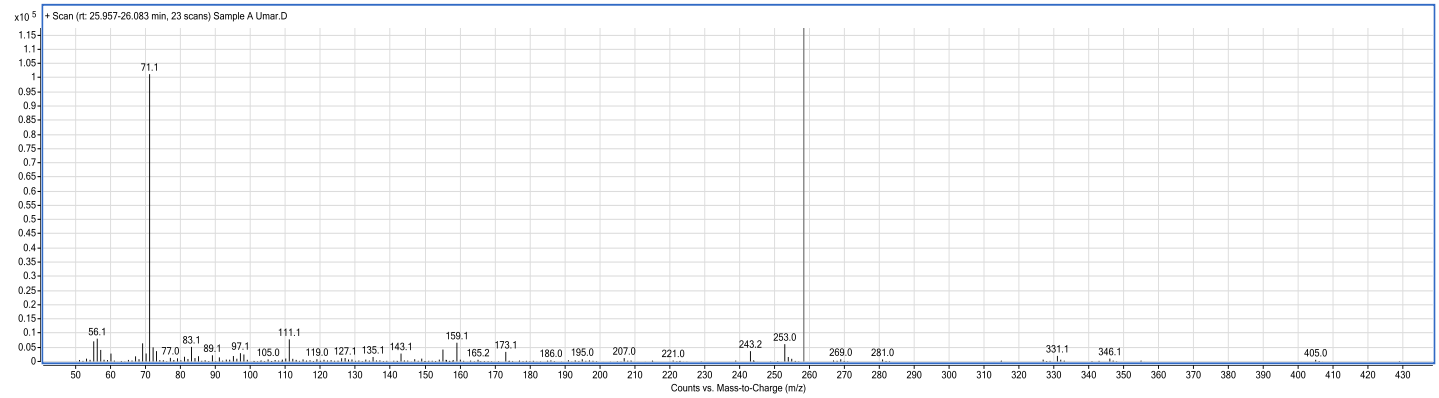




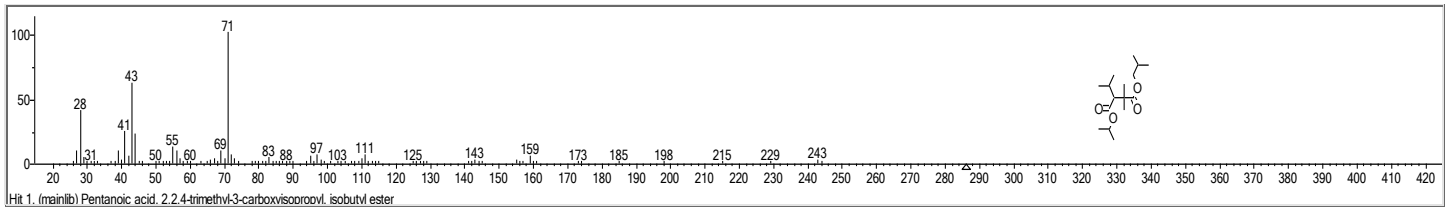
peak 21, rt: 25.448, base peak 121.0 Mz 405.1

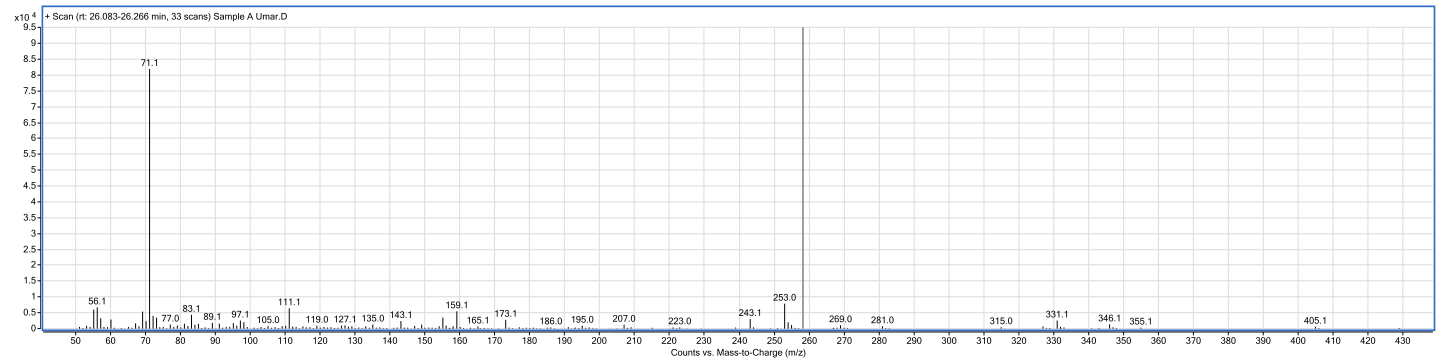


peak 22, rt: 26.043, base peak 253.0 Mz 405.1

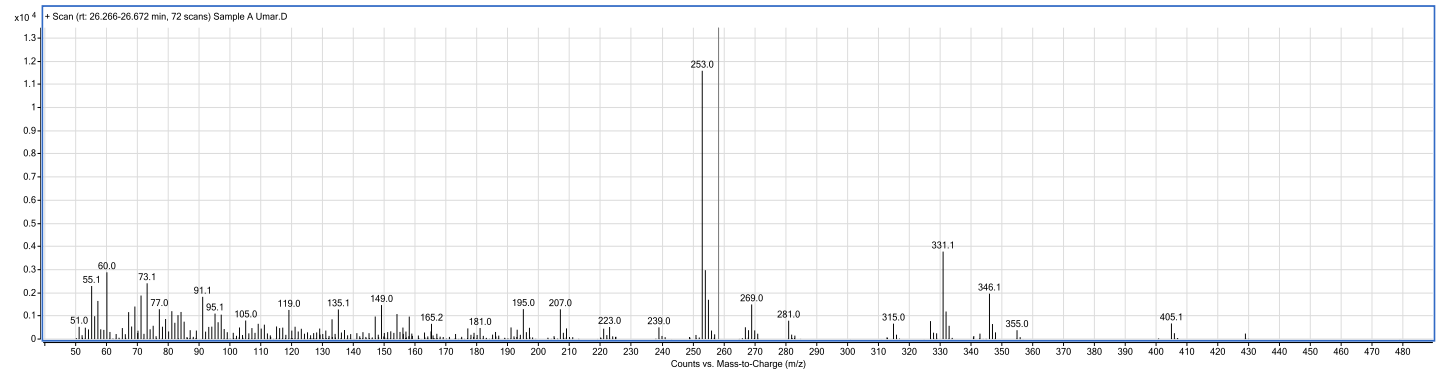


peak 23, rt: 26.140 mins, base peak 71.1 Mz 405.0

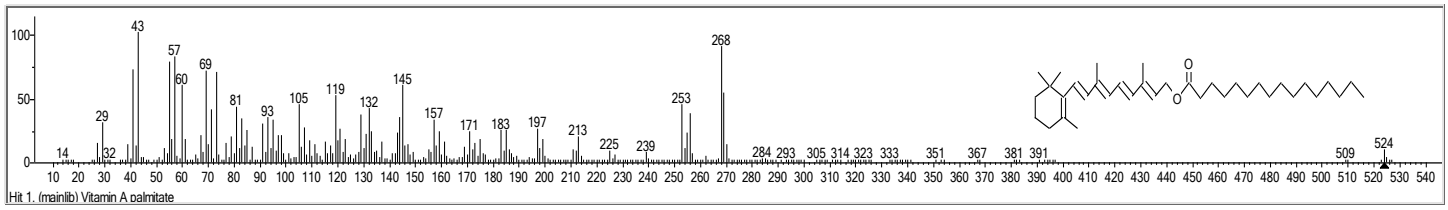


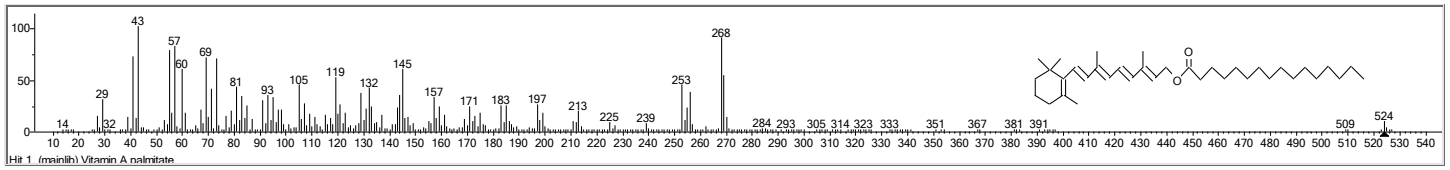


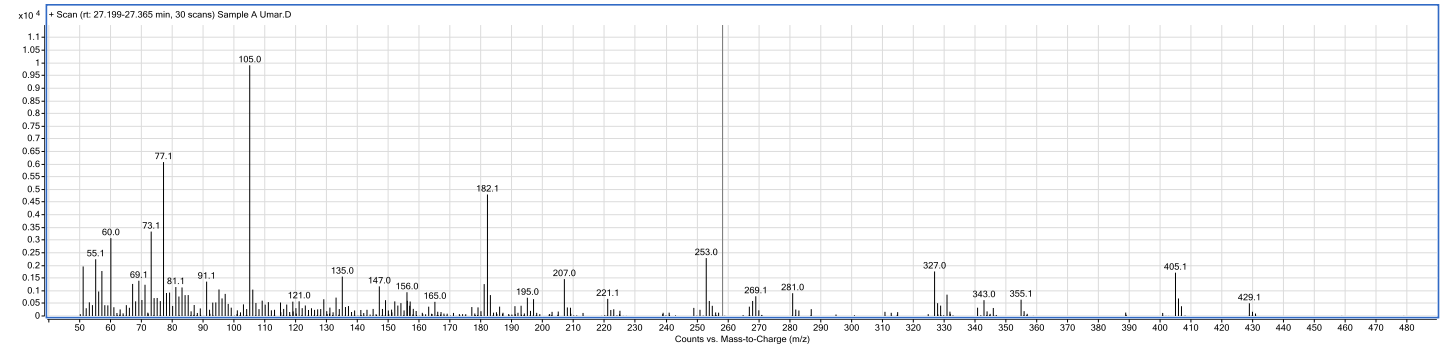
peak 24, rt: 26.392 mins, base peak 71.1 Mz 405.0



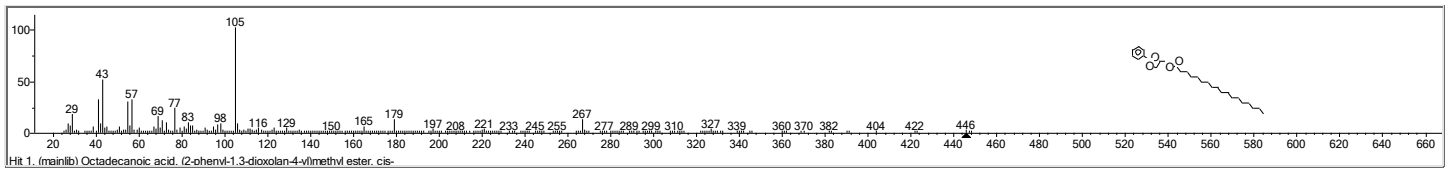
peak 25, rt: 27.290mins, base peak 253.0 Mz 405.1

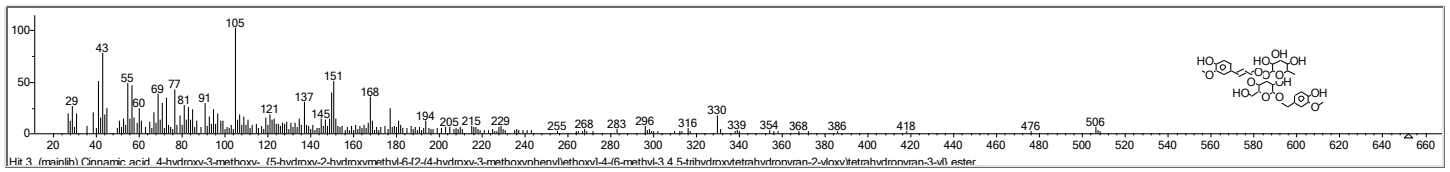


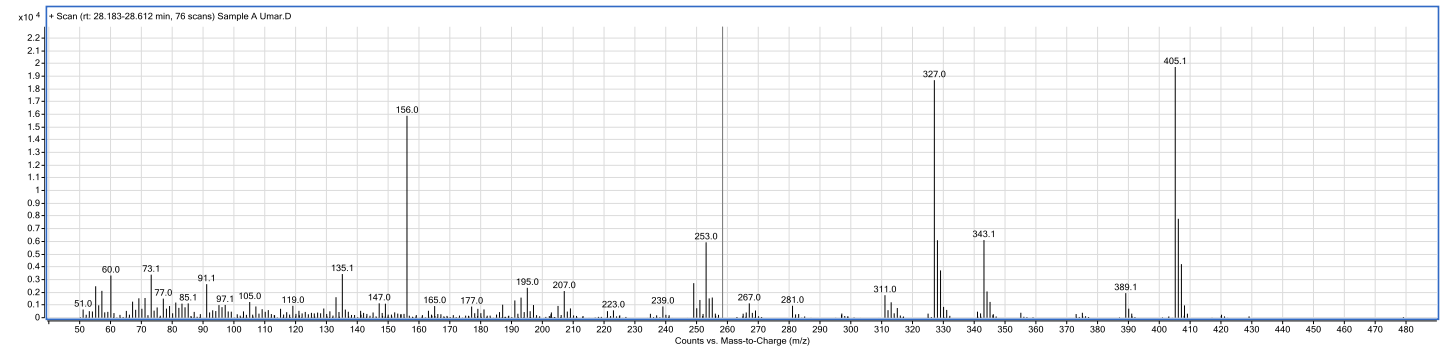




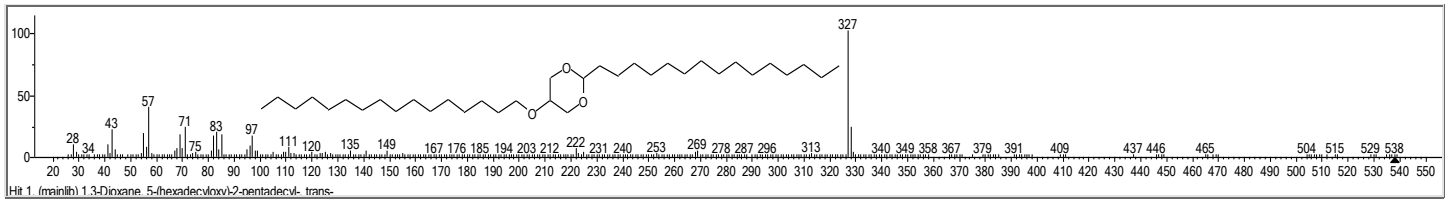
peak 26, rt: 28.400, base peak 105.0 Mz 429.1

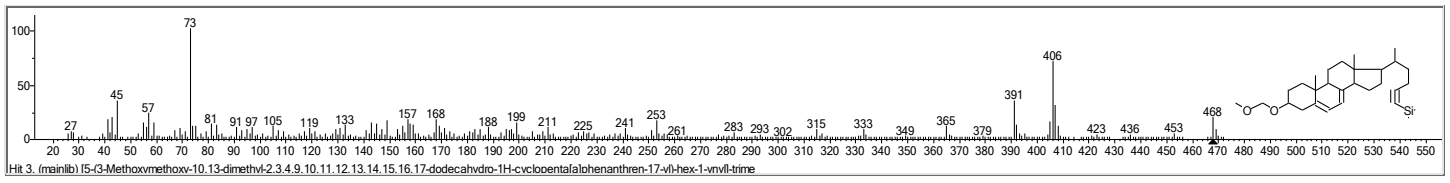


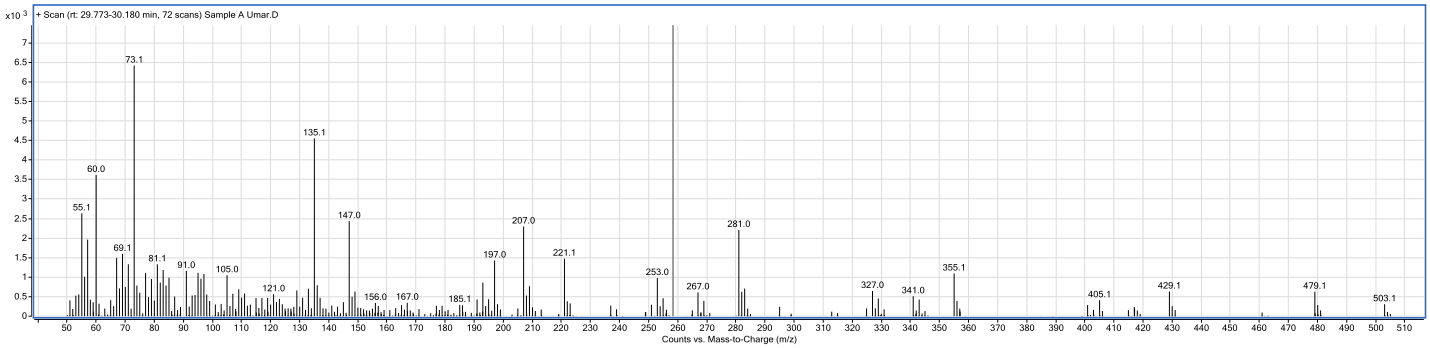




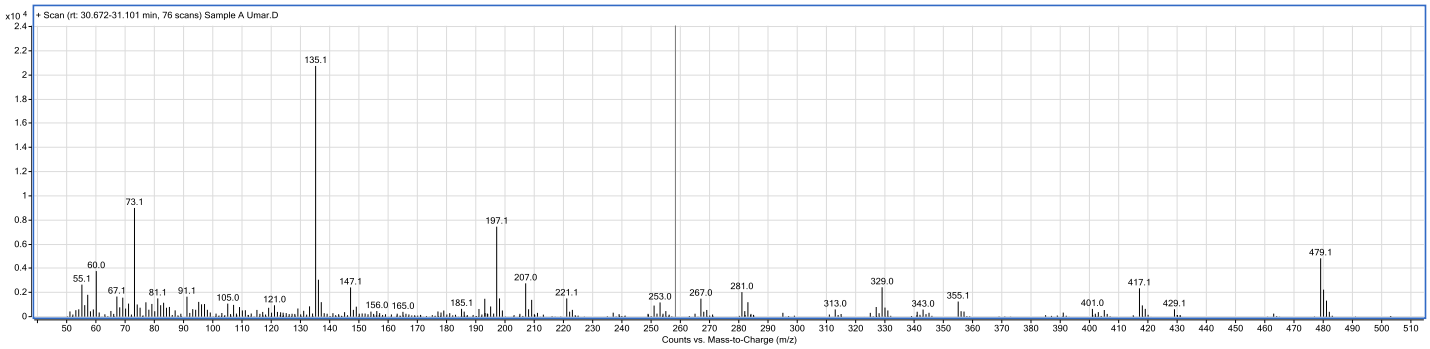
peak 27, rt: 29.991, base peak 405.1 Mz 405.1



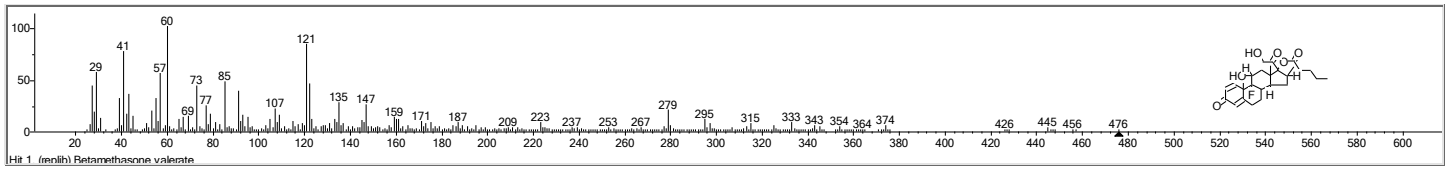


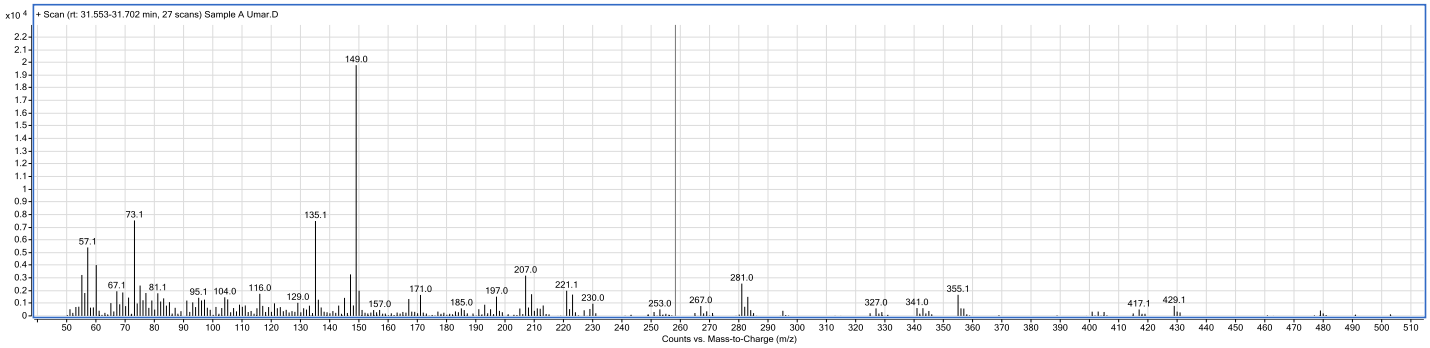


peak 28, rt: 30.906, base peak 73.1 Mz 503.1

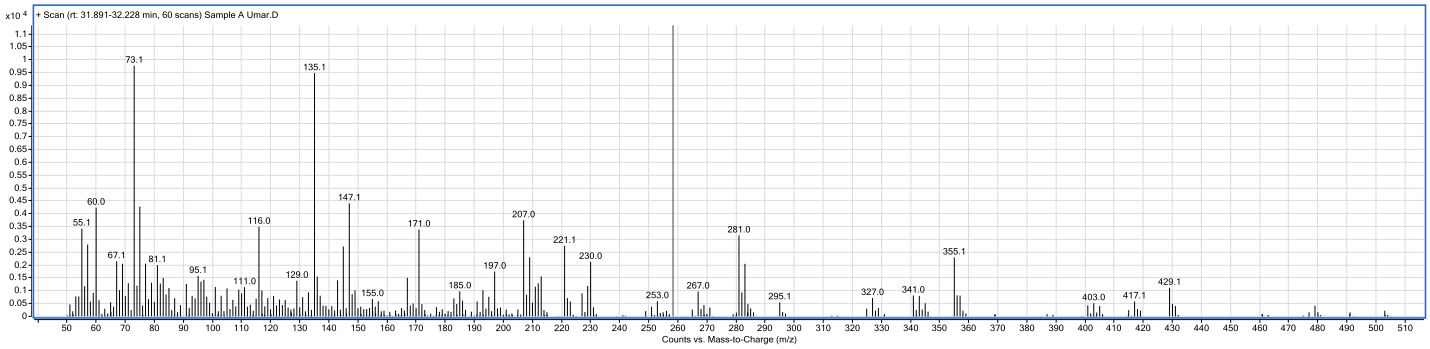


peak 29, rt: 31.639, base peak 135.1 Mz 479.1



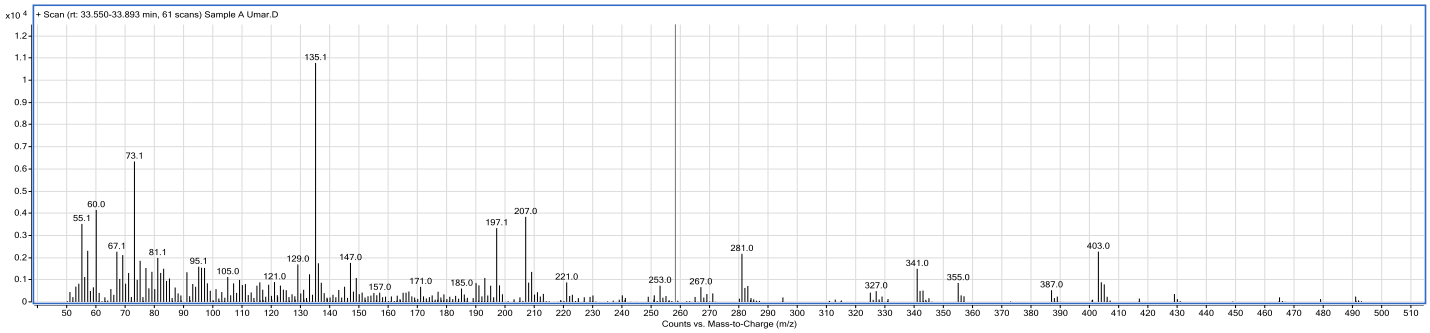


peak 30, rt: 32.091mins, base peak 149.0 Mz 429.0

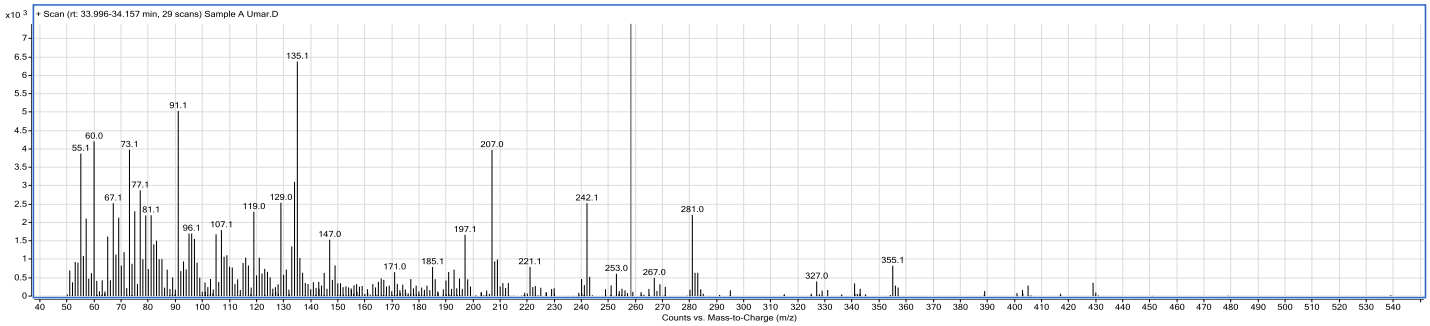


peak 31, rt: 32.377, base peak 73.1 Mz 429.0

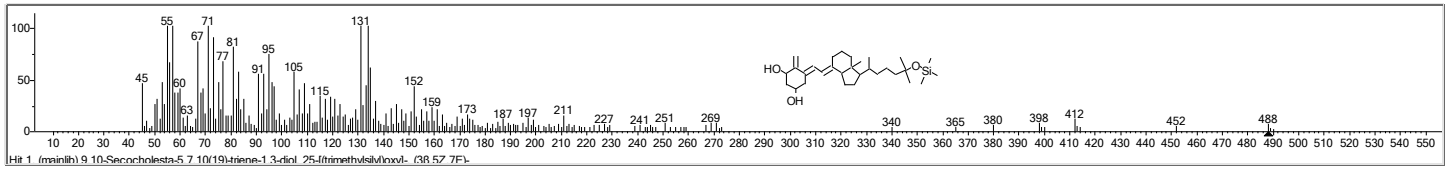
peak 32, rt: 32.675, base peak Mz

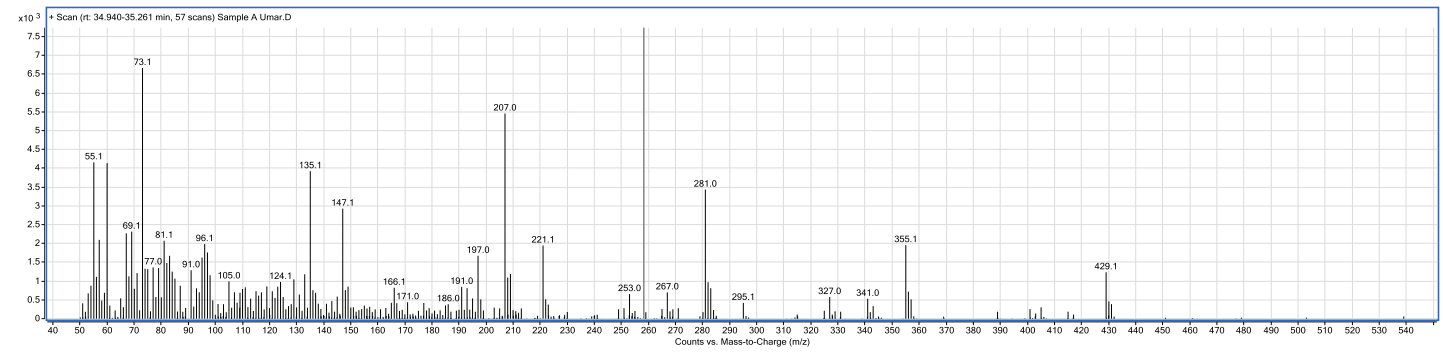


peak 33, rt: 33.739, base peak 135.1 Mz 403.0

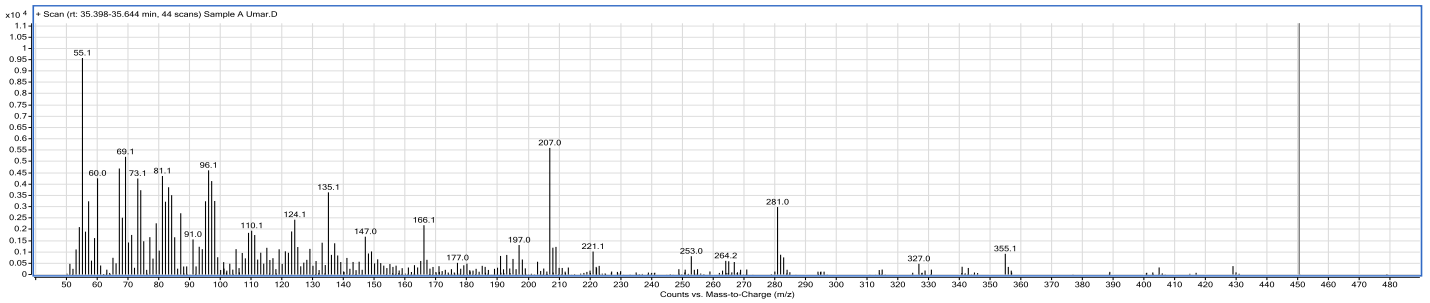


peak 34, rt: 34.071, base peak 135.1 Mz 355.1

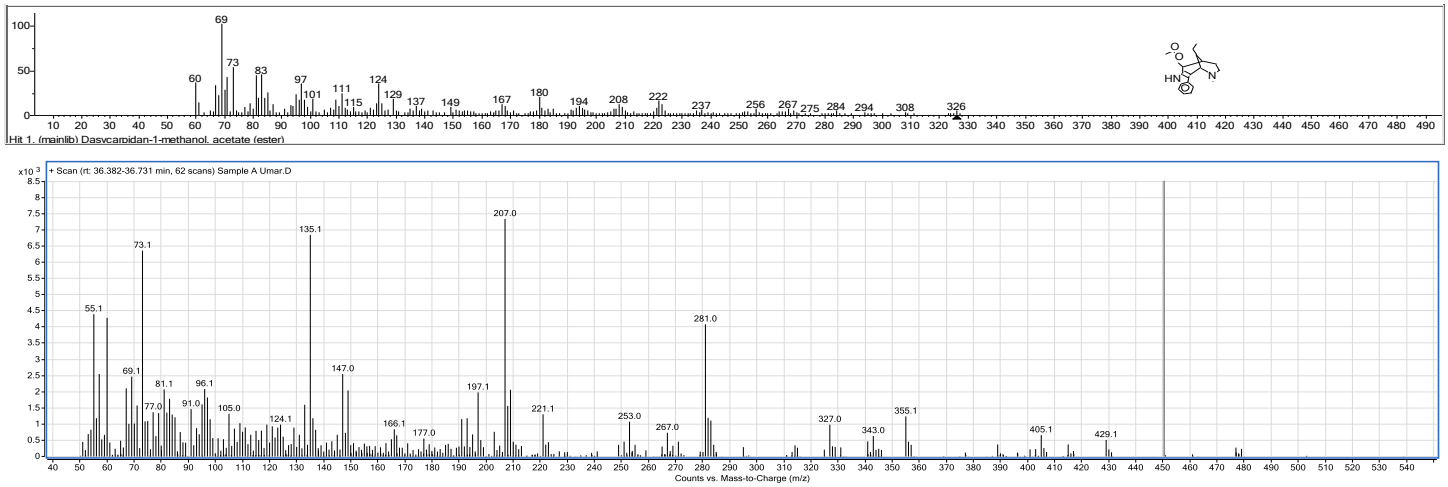




peak 35, rt: 35.141, base peak 73.1 Mz 429.0

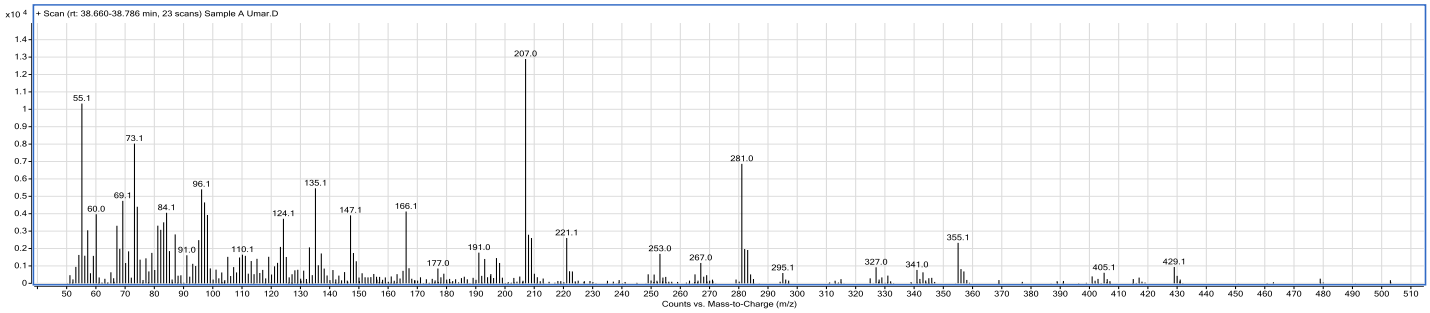


peak 36, rt: 35.530, base peak 55.1 Mz 355.1

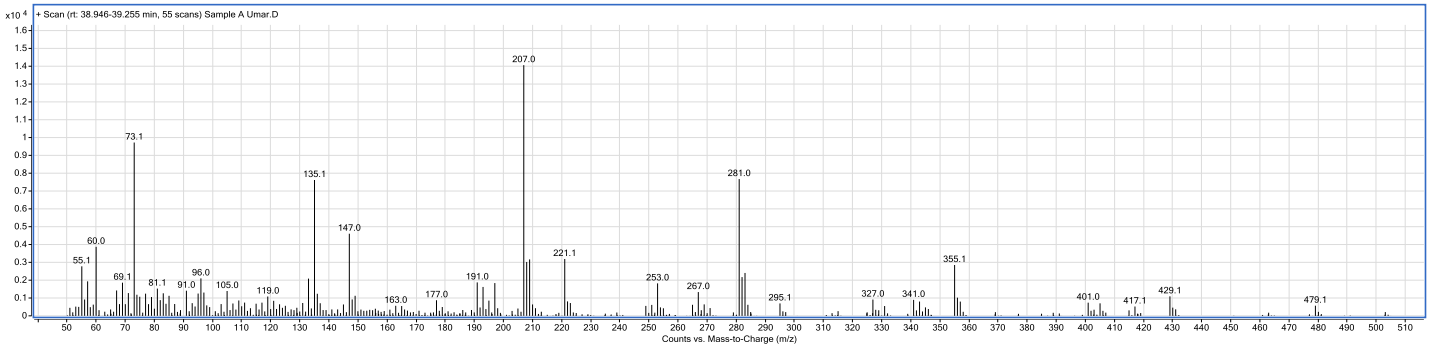


peak 37, rt: 36.531, base peak 207.0 Mz 429.1

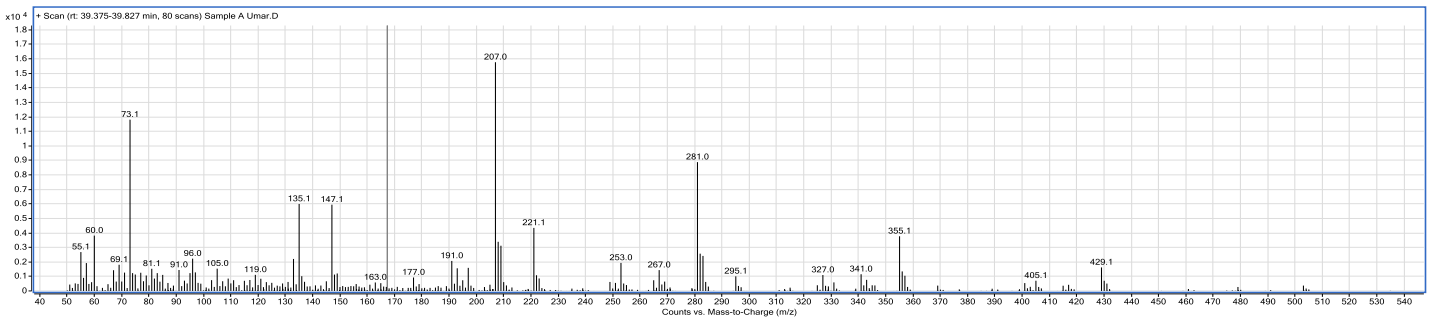
peak 38, rt: 37.389, base peak Mz



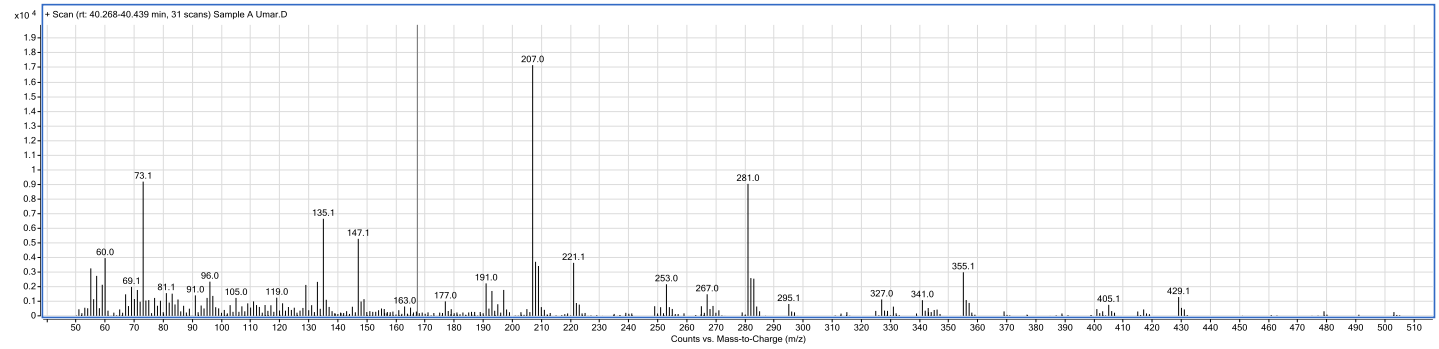
peak 39, rt: 38.723, base peak 207.1 Mz 429.0



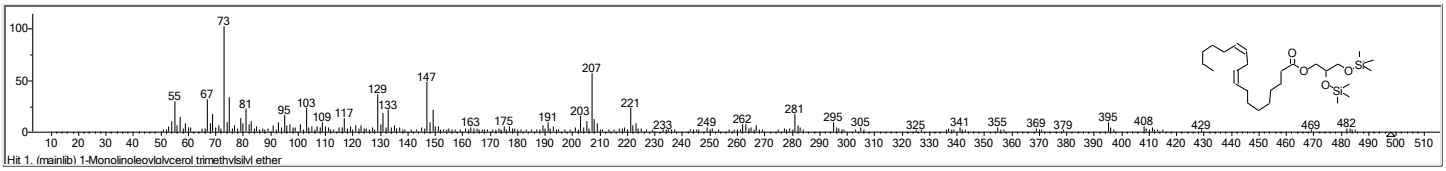
peak 40, rt: 39.117, base peak 207.1 Mz 479.0

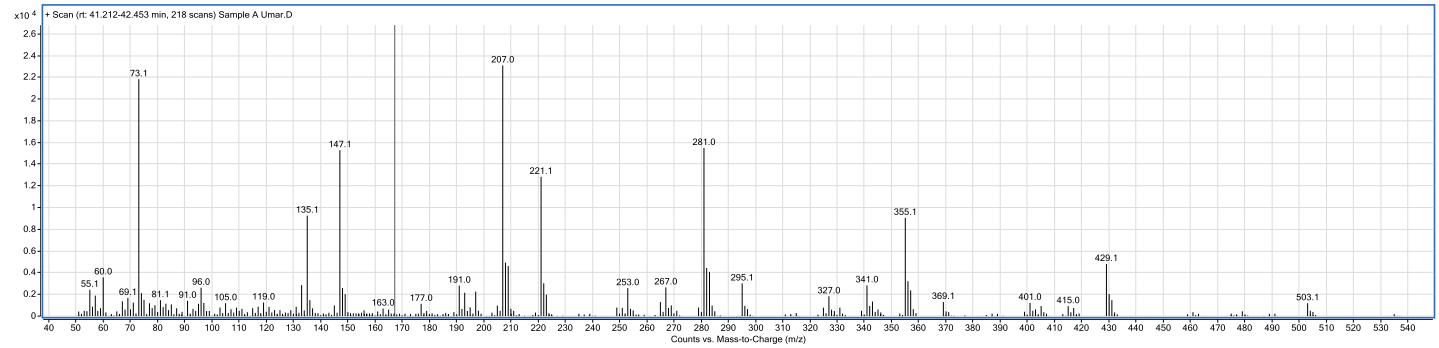


peak 41, rt: 39.552, base peak 207.0 Mz 429.1



peak 42, rt: 40.382, base peak 207.0 Mz 429.0





peak 43, rt: 41.812, base peak 207.1 Mz 503.0

IV. CONCLUSION

In conclusion, the crude extract and the partitioned portions revealed the presence of some medicinal compounds such as carbohydrate, cardiac glycosides, flavonoid, terpenoid, saponins, alkaloid and tannins. GC-MS analyisis revealed the presence of many compounds with therapeutic values.

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