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Phytochemical Profile of *Urtica dioica* L. by gas chromatography-mass spectrometry GC-MS and antioxidant activity.

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Abstract. *Leaves are rich in many phytochemical compounds, all bioactive compounds formed normally during plants metabolism as secondary products. The phytochemical compounds screened by gas chromatography-mass spectrometry (GC-MS) method. Twenty nine bioactive phytochemical compounds were identified in the . The GC-MS analysis of U .dioica revealed the identification of phytochemical compounds is based on peak area, and retention time. Also determine of antioxidant activity in leaves. The first compound was benzene that presence at 5.339 min with peak area 1.18, while the 9-Octadecenamide was the last compound shown at 68.304 min with 2.18 peak area. Including organic compounds, fatty acids and phenols. A DPPH radical scavenging e results showed U. dioica extract at 600 concentrations high than ascorbic acid was93.76 µg/ml while aceorbic acid was 91.14 µg/ml. this finding indicate that U. dioica have high antioxidant ability. . This manuscript to cover the chemical composition of methanolic extract of U. dioica leaves (stinging nettile) that growing naturally in Iraq, as a chemical study and provide details of pharmacological propos*

Keywords: *Biological Activity, Chemical Profile, Phenolic Acids, Polyphynolic Compounds, And Stinging Nettle*

INTRODUCTION

" Stinging Nettle" is the common name of genus *Urtica* L. (Urticaceae). *Urtica* genus including over 80 taxa distributed globally. The genus is knowing as a weed, but can occupy a range of natural habitats. Many species are relatively wide spread . *Urtica* genus used a lot by human as food and other aims, such as productin of fiber, or food for animal, and phytoremediation [1]. *Urtica* known as a medicinal plant with diversified health advantages. Many of taxaof this genus has a pharmacological effect commonly used for treat anemia, asthma, coughs, dandruff, diabetes, diarrhea, eczema, fever, gout, hemorrhoids, bleeding of nose, prostate hypertrophy, rheumatism , sciatica, scurvy, snake bites, and tuberculosis [2], moreover the stiff hairs leaves are that covered both sides of leaf produce hot sensation when touched , histamine and acetylcholine released as a biochemical mediators, from the hairs, which act like needles (Grauso et al., 2020) this property used to urtication (external stinging), in which fresh stems and leaves are applied locally to treat pain of joint [3]. [4] A lot of progressive studies cirtens the medicinal use traditionally by several agricultures and cleared the anti-inflammatory , antioxidant activities, antiviria, antimicrobial, antihelmintic, anticancer,

nephrotective, hepatoprotective [4]. (Mutke et al. ,2014; Kumar et al., 2017). Several bioactive compounds settle down in leaves [5]; [6]. *Urtica* wealthy by bioactive compounds can isolated from it, for example amino acids, carotenoids, fatty acids, and phenolic acids. Their extracted are used for nutritional properties, anti-inflammatory and antioxidant factors [7].

Besides the basic substances, . Naturally, presence phytochemicals structures considerable a many of compounds of promoting health, such as fatty acids, phenolic compounds [8]. Phenolic compounds recognized by their antioxidant activity, antioxidant system make cells defends against the toxic effects thereby preventing peroxidation of lipids [9]. The plant is described as a natural antioxidant when it possesses flavonoids and phenolic compounds[10]. Significantly increased the attention of the scientific community over the last several decades of biological activity compounds due the broad range has charmed due their safe effects on human health [11].

MATERALS AND METHODS

a. Collect plant samples

Samples were collected from seven districts in central Iraq during the period March-May (2024) and the plants were classified by a BUE herbarium. The mature leaves were dried and preserved for study.

b. Preparation of Alcoholic plant extracts

100 ml of 70% ethyl alcohol and 10 g of investment husks base was added to it .The incubator of the shaker of plant, and its pusher, the incubator of the shaker, at a temperature of 35 °C for a period of time then filter the position of using filter paper (What man No. 1), Centrifuge for 10 minutes / 3000 revolutions per minute using the Rotary Evaporator, after placing the extracted product in clean, pot at 4°C until use [12].

c. GC-MS Analysis

A number of active compounds were identified in the plant using the gas chromatography with mass spectrometer (GC Mass) as shown in figure 1 with Agilent model 6890 coupled with mass spectrometer, model 5973N, with HP-5MS capillary column with static phase of 5% methylphenyl siloxane (length 30 meters) , the inner diameter is 0.25 mm and the thickness of the resident layer is 025 um and the ionization energy is 70 electron volt. The quadruple temperature was150 °C, and the solvent delay time was 3.5 min. the constituents were identified by comparing their mass spectra with those of NIST library data for the GC-MS system, instrument Conditions: Gas Chromatograph: ,Injection volume 1µl, Pressure 11.933 psi, GC Inlet Line Temperature: 250 ˚C, Aux heaters Temperature 300 ˚C, Carrier Gas: He 99.99%, Injector Temperature: 250 ˚C Scan Range: m/z 25-1000, Injection Type: Splitless, Oven Program: Temperature, Ramp 1 60 ˚C hold to 3 min., Ramp 2 60 ˚C to 180 ˚C, 7 ˚C/min, Ramp 3 180˚C to 280˚C, 8 ˚C/min, Ramp 4, 280˚C hold to 3 min.

d. Antioxidant activity determination

Antioxidant activity were measured in the leaves based on DPPH radical scavenging assay according to (Alamgir et al., 2014) with some modification. We prepared different concentrations were 40, 80, 160, 320, 400, 600, 800, 1600, and 2400 mg L^{1-}, the samples of leaf extract solution were diluting with 80% methanol. The 2ml was standard for each concentration level, was mixed with 31.2 mg L^{1-}. Methanol solution of DPPH was incubated for 5 minutes, after incubation the solution volume was adjusted to 5 ml with 80% aqueous alcohol with room temperature incubated for 30 minutes. Resulting solution was absorbation measured at 517nm compering with a reagent blank. The radical scavenging activity was calculated as following equation, scavenging activity % = A-B/AX100 [13].

Where A is pure DPPH solution absorbance, and B is the presence DPPH solution in sample absorbance. As standard we used the ascorbic acid. The antioxidant activity determination were expressed as ml of ascorbic acid equivalents per g (mg AAEg $^{1-}$) dry specimen.

RESULTS

The GC MASS analysis bring to light the presence 29 bioactive compounds (all data in fig 1.to 30) in methanol extract of *U. dioica* leaves. the first compound was benzene that presence at 5.339 min with peak area 1.18, while the 9-Octadecenamide was the last compound shown at 68.304 min with 2.18 peak area. The study revealed the organic compounds such us benzene, p-Xylene , Mesitylene , Cyclohexanol, Nonadecane, Eicosane, Heptadecane hydro carboxylic acids like 1,2-Benzenedicarboxylic acid, Phthalic acid, Hexadecanoic acid, and Eicosane, our results agree with (Đurović et al., 2017) The appearance of these compounds indicates the fall of a series of acids as a result of high temperatures, which led to the loss or transformation of many of them, as the ideal temperature for the appearance of acids is 40°C [14]. The fatty acids showed in the analysis such as Butanoic acid, Pentadecanoic acid, Decanoic acid, and Octadecadienoic acid, this data compatible with (Durovic et al., 2024) that mention fatty acids showed and its percentage ranges between 30-40% depending on the plant part and the stage of maturity and phenols such as isoeugenol 1, Phenol, 2-methoxy-4-(2-propenyl), H-Indene, 2,3-dihydro-1,1,3-tri [4]. Schinifoline, this is what scientists have explained about the presence of phenols in plants [15], [16]. No gallic acid and no catechin in the parts of plant [17], which is in agreement with other studies [18] but contrary to other studies that reported the presence of only gallic acid [6] or both compounds [19] The results showed that leaves were the richest in polyphenolic compounds, followed by stalk and root [17], The precence of phenolic compounds makes plant become natural antioxidants [7] compounds with pigment as Phytol, this result agree with [4], The presence of the dye indicates that the solvent is the best way to separate it, taking into account the extraction conditions. The presence of the dye is related to the geographical area, season and plant development, so it is manufactured with this efficiency.

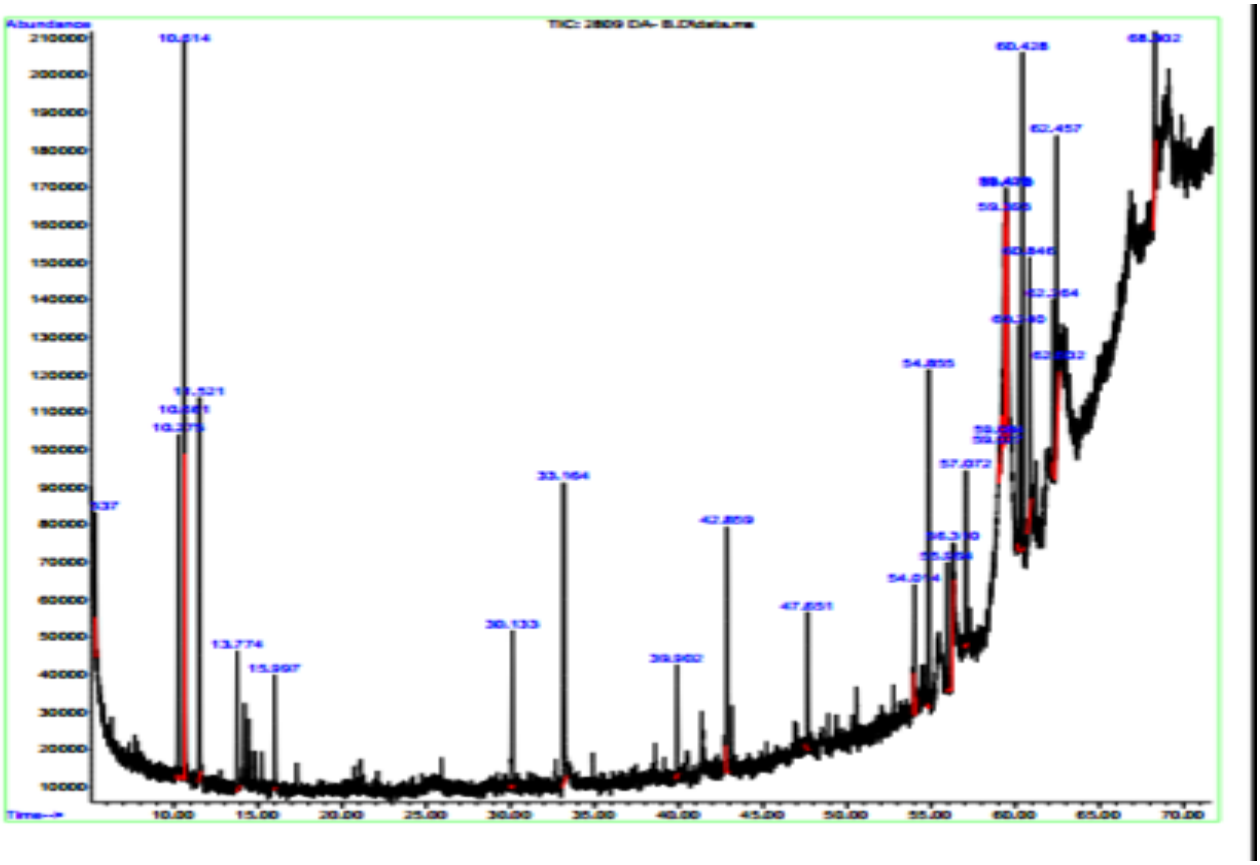


Figure 1. GC MASS analysis in methanolic extract of *U. dioica* leaves

The compound started to appear at the 5.339 minute and ended at the 68.304 minute as showed in figure 1 and the evidence was identical to the compounds started with 18 % to 99% . our result to the photochemical compound agree with Omer and Mohammed (2023) that cleared the potential source of phytochemicals be a good tool in identification of alkaloids absence in pharmo standards. Our results showed absence of alkaloids this outcome agree with (Joshi & Uniyal, 2017) who noted in ethanol and ethyl acetate presence of flavonoids, phenols, and tannins, but lack of alkaloids) [20]. The phytochemical analysis of *Urtica* leaves showed differences in the content of bioactive compounds among five solvents used [21]. To understanding the profile of bioactive significance the researchers forced to deal with various analytical techniques [22], Anywise, the outcome it changes due to many reasons, including diversity of environment, changes of geographic, the plants' part that used in extraction such as root stem, leaf or flower, technique of extraction, and the solvent that employing in extraction [7], [9], [19], [23].

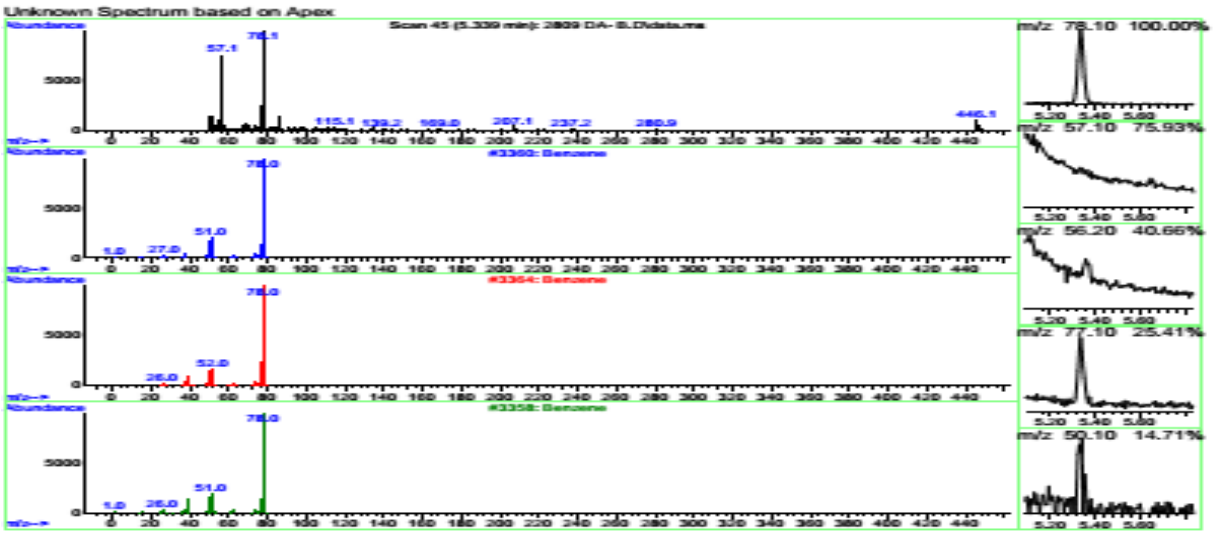


Figure 2. The revealed compounds in (RT)= 5.339

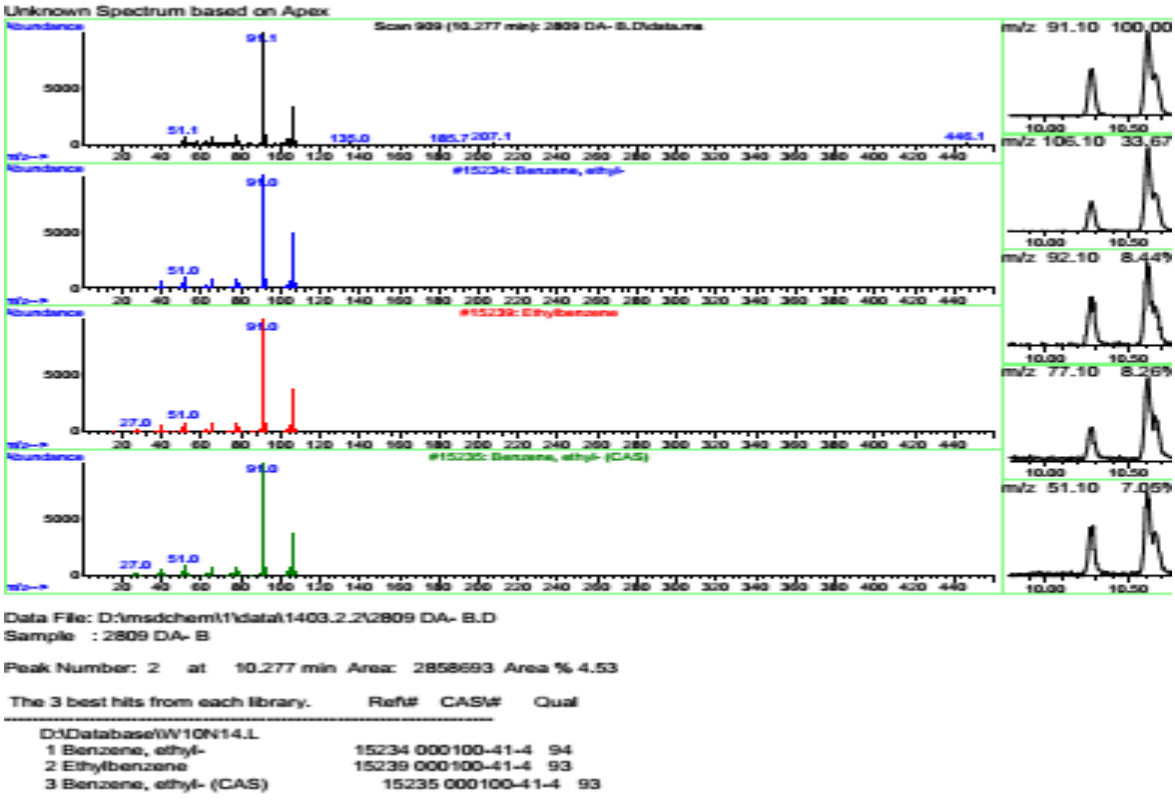


Figure 3. The revealed compounds in (RT)= 10.277

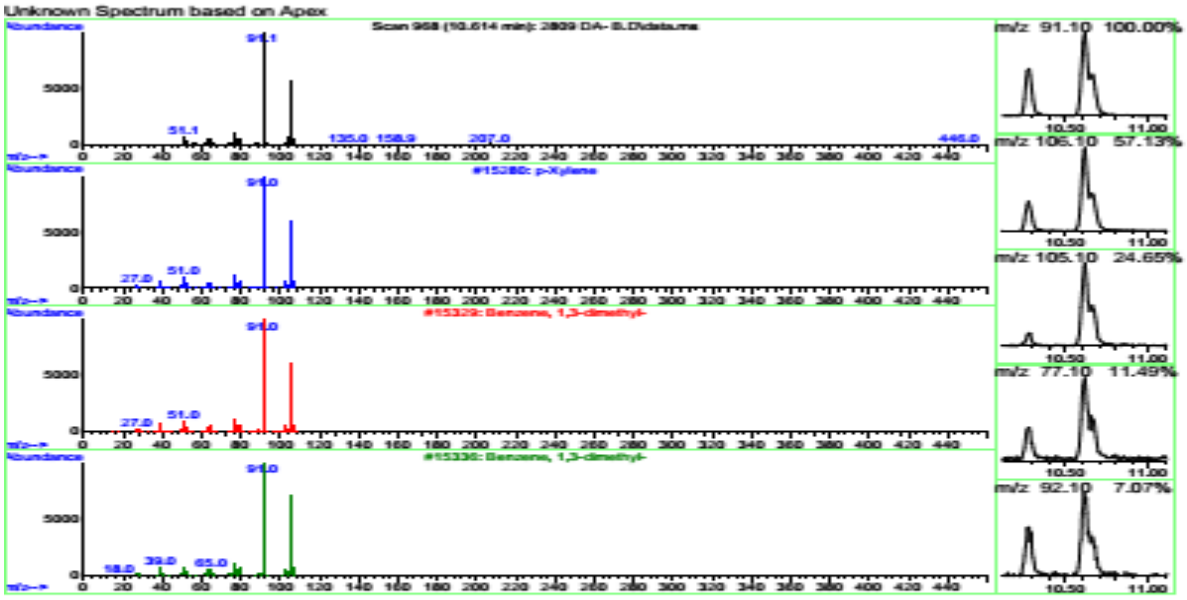


Figure 4. The revealed compounds in (RT)= 10.614

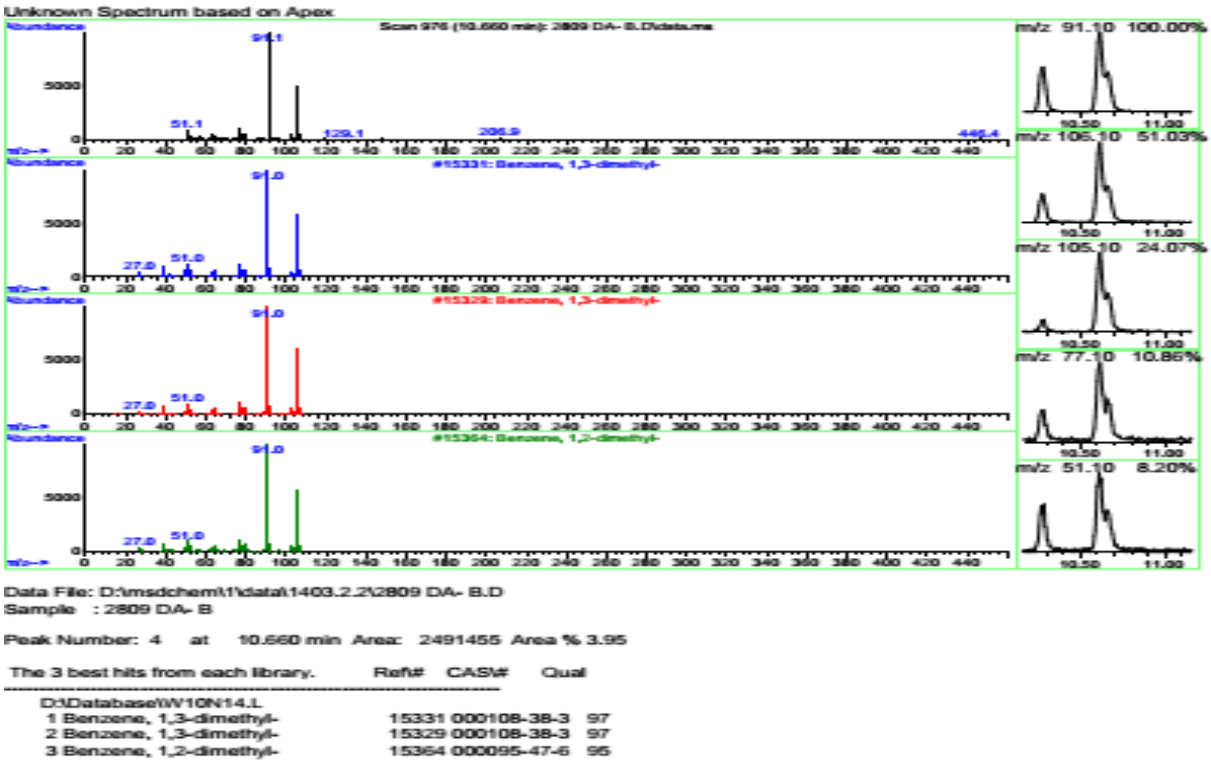


Figure 5. The revealed compounds in (RT)= 10.660

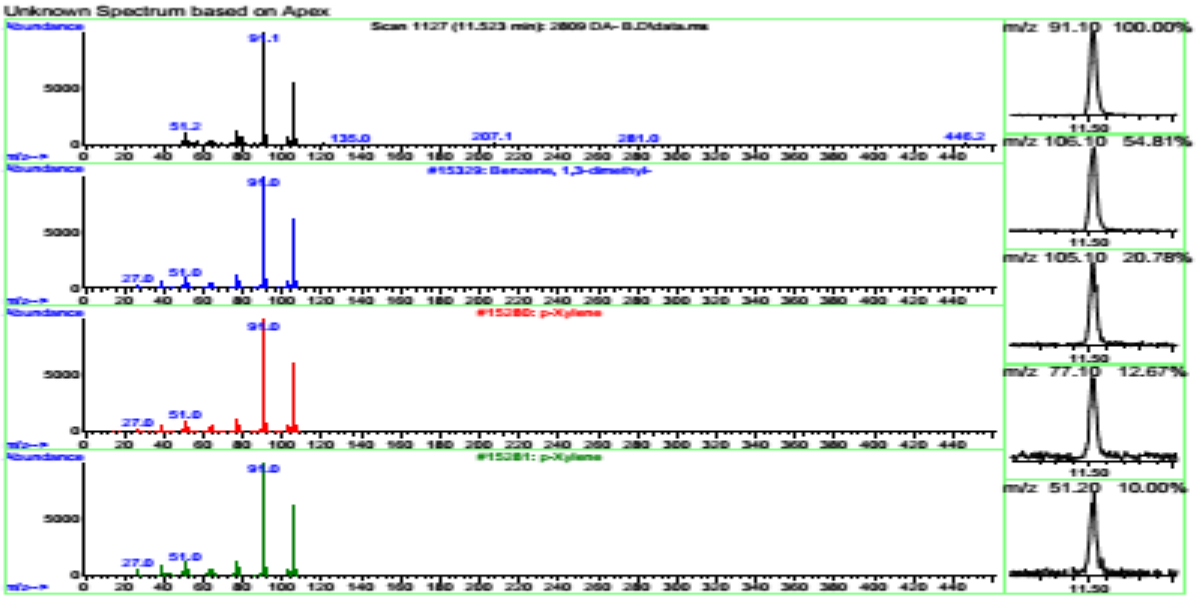


Figure 6. The revealed compounds in(RT)= 11.523

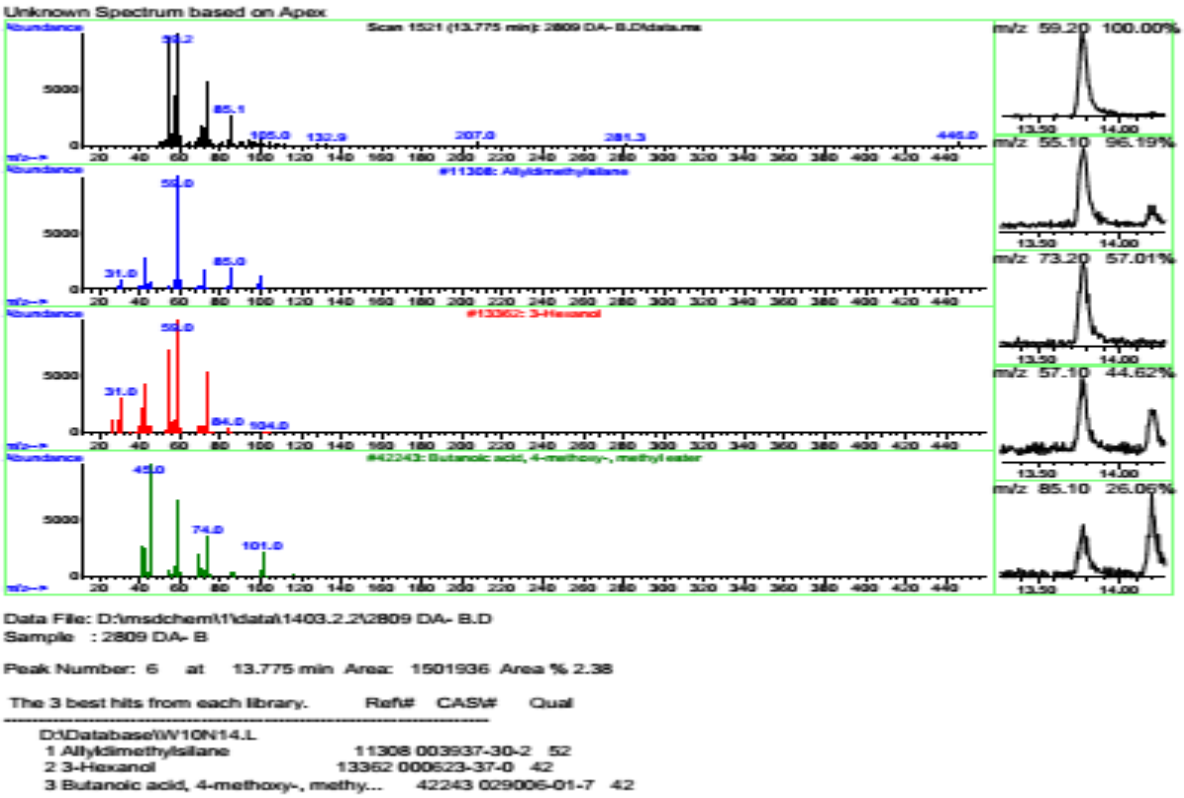


Figure 7. The revealed compounds in (RT)= 13.775

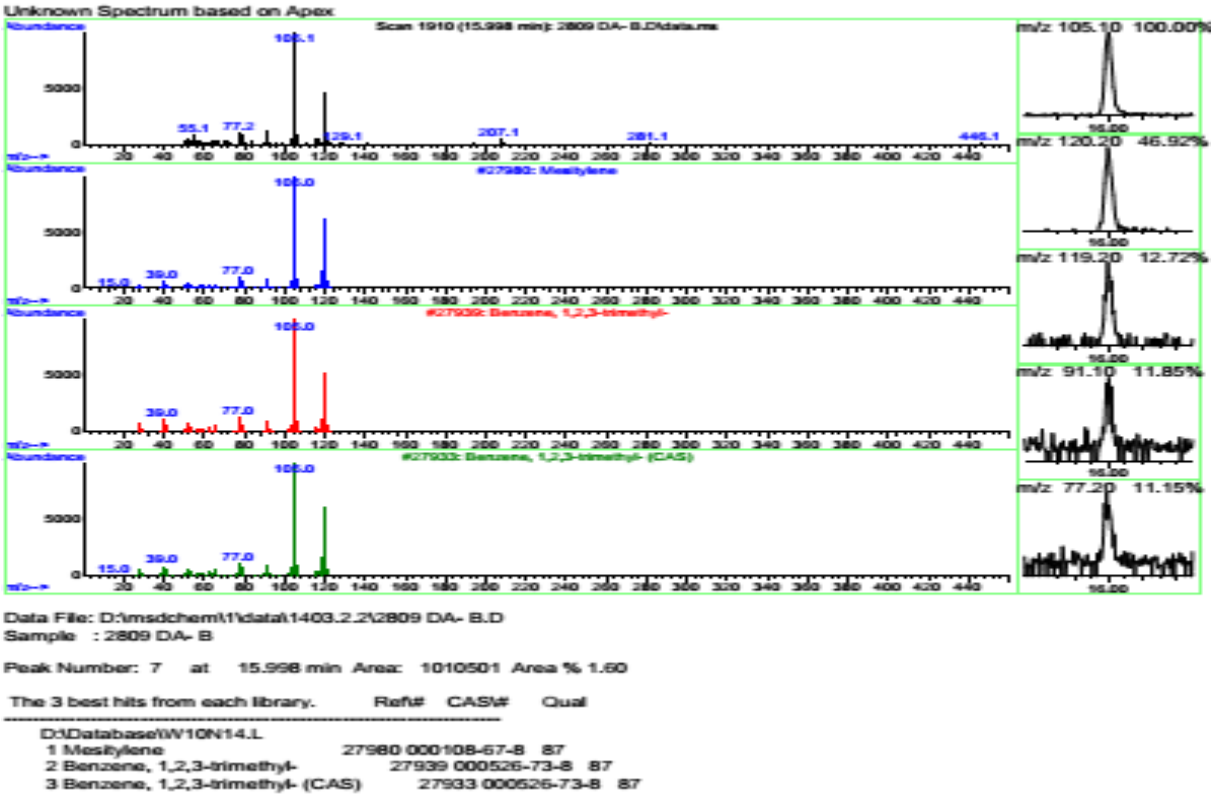


Figure 8. The revealed compounds in (RT)= 15.998

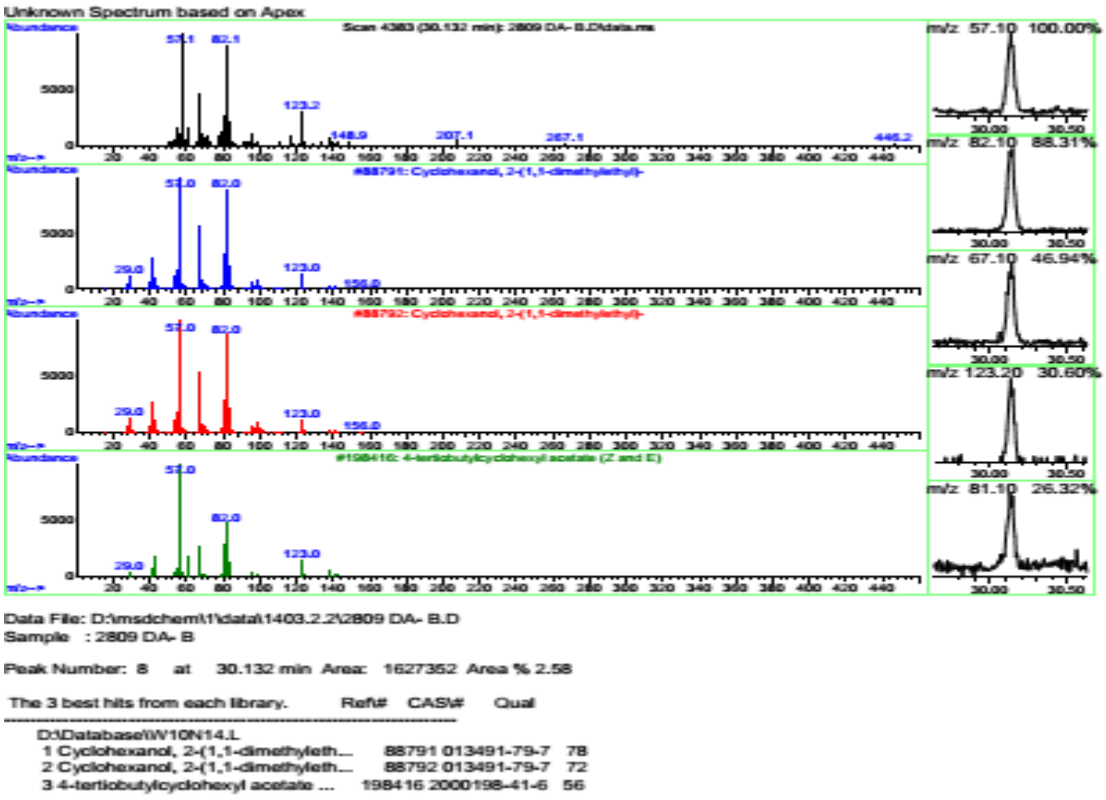


Figure 9. The revealed compounds in (RT)= 30.132

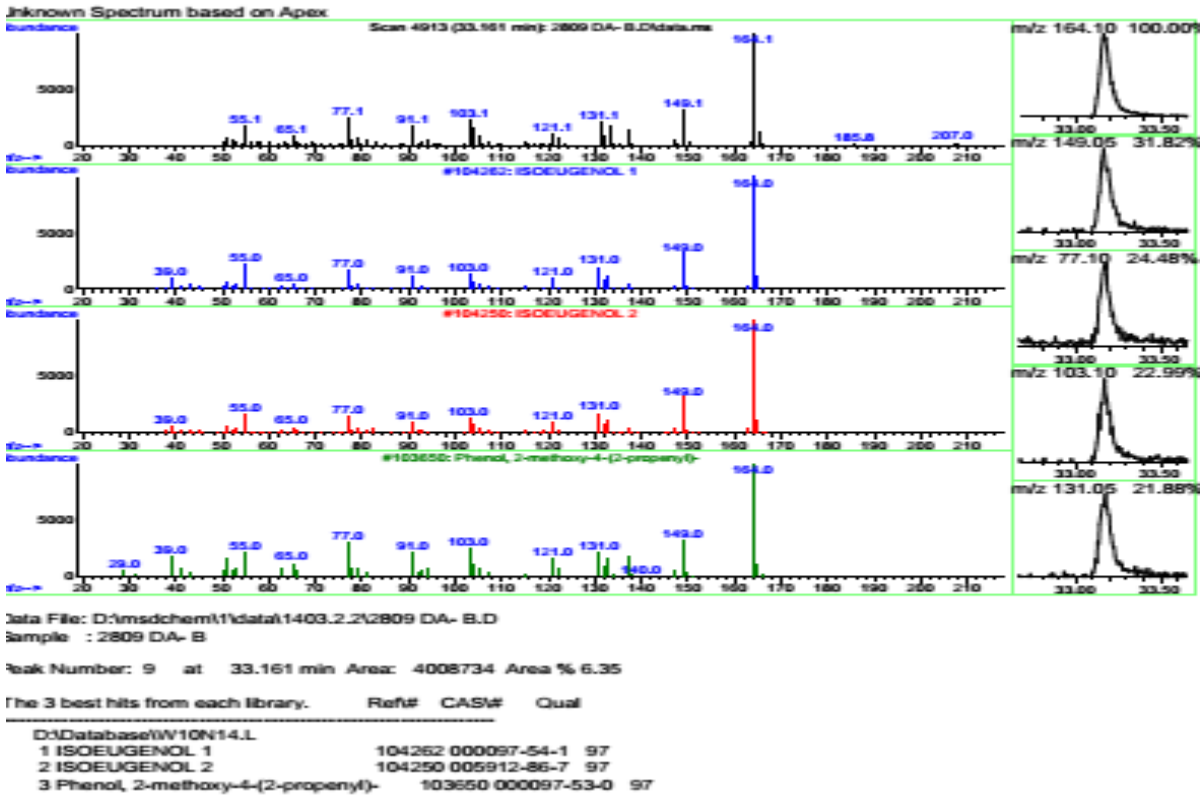


Figure 10. The revealed compounds in (RT)= 33.161

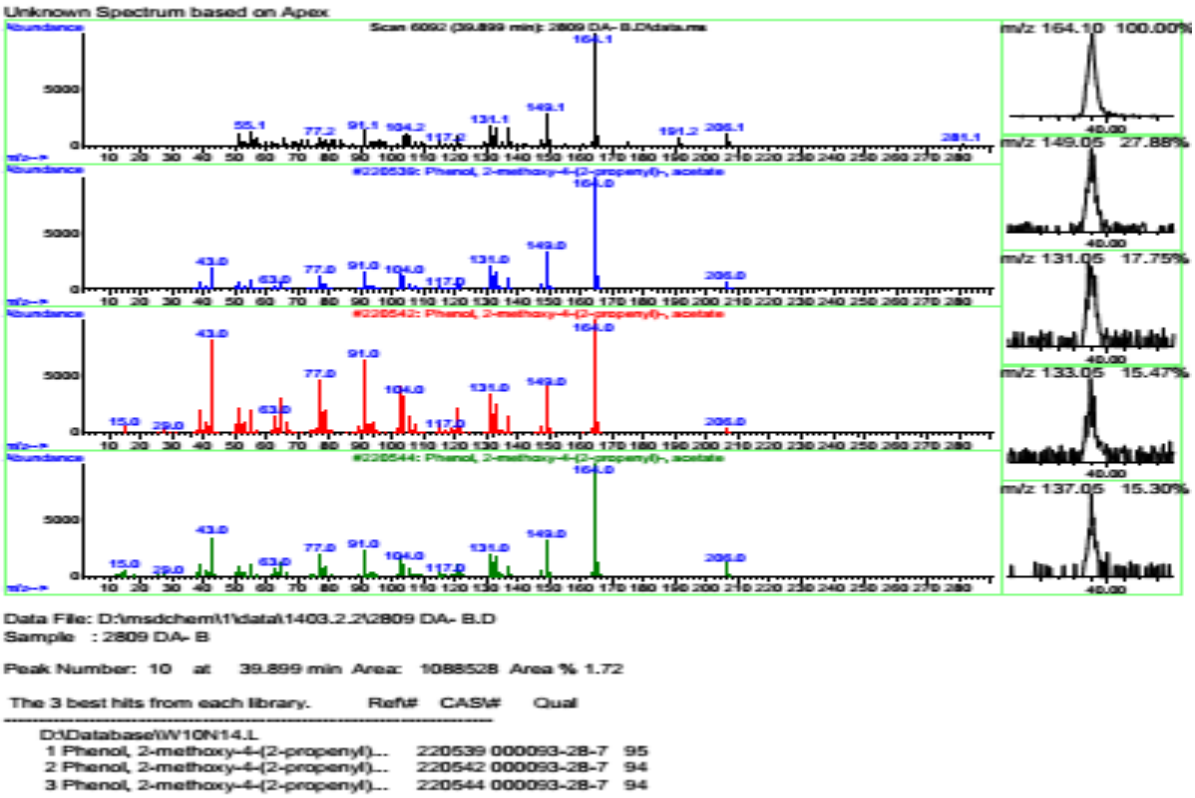


Figure 11. The revealed compounds in (RT)= 39.899

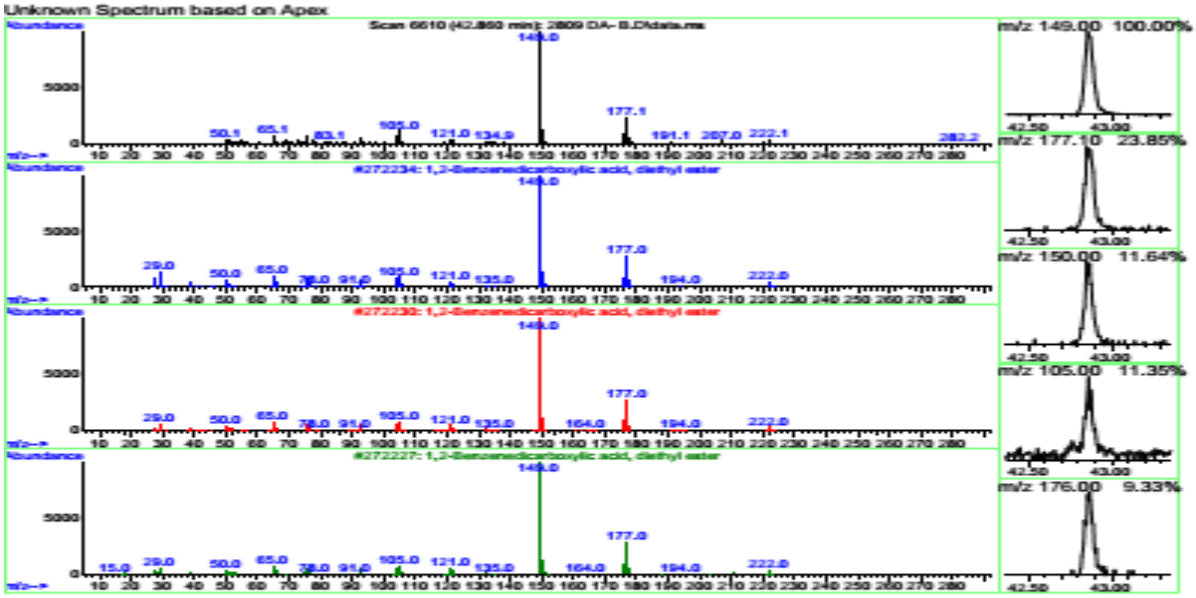


Figure 12. The revealed compounds in (RT)= 42.860

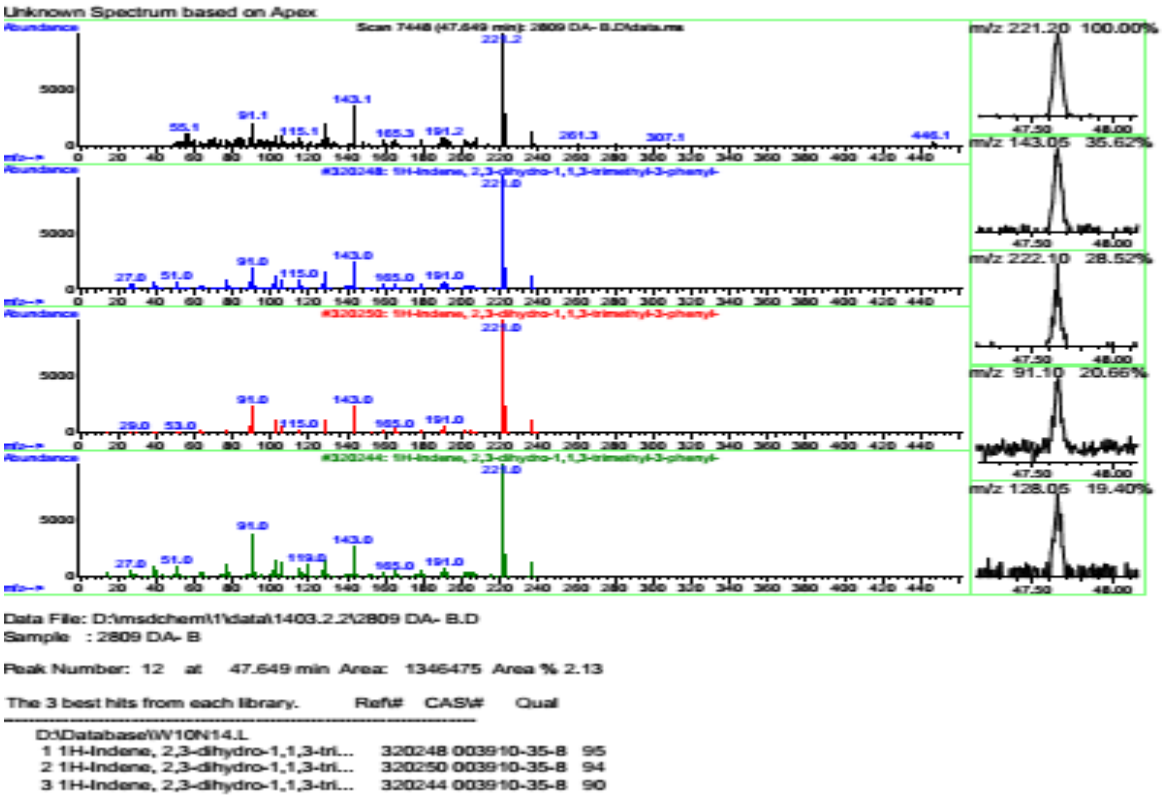


Figure 13. The revealed compounds in (RT)= 47.649

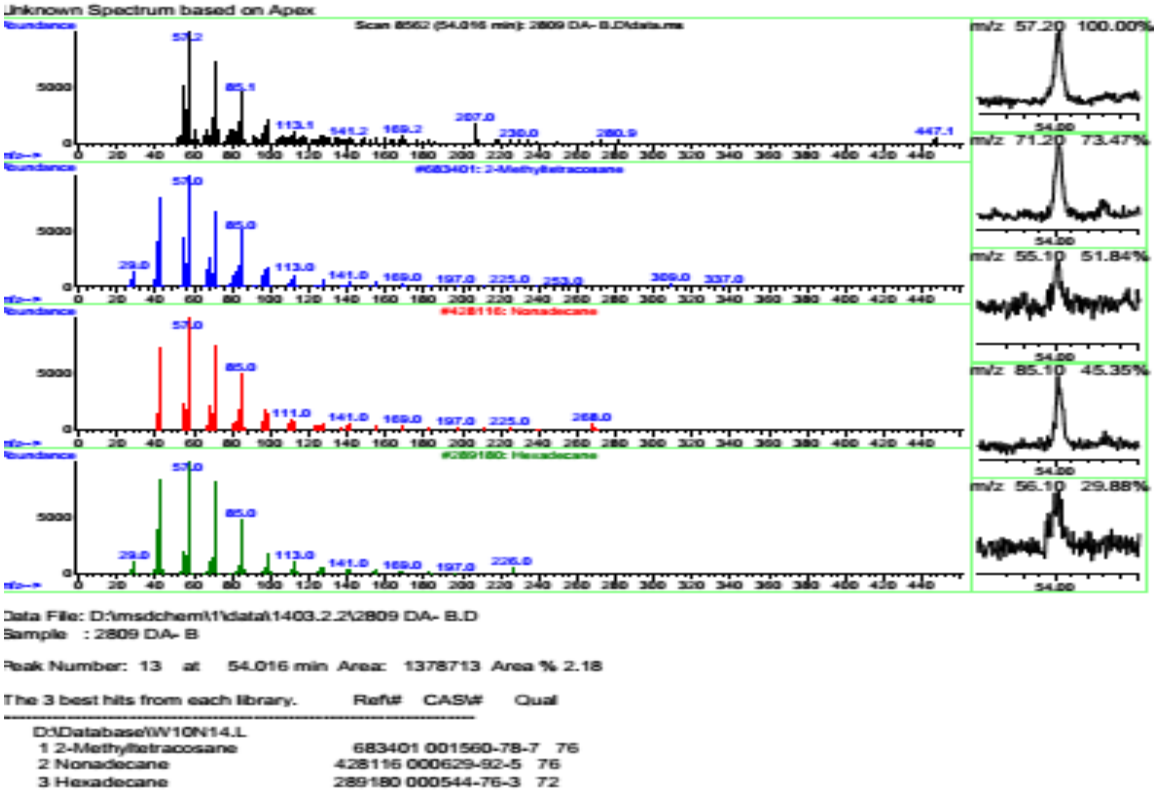


Figure 14. The revealed compounds in(RT)= 54.016

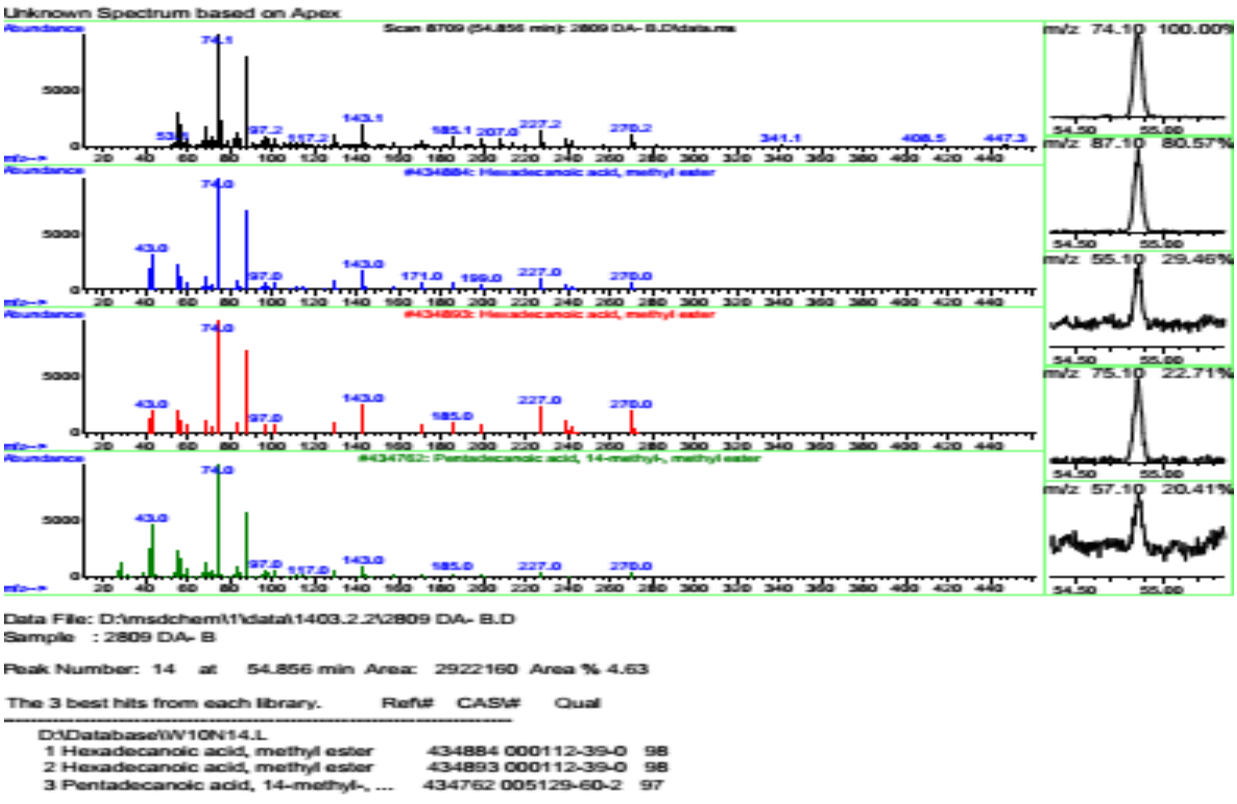


Figure 15. The revealed compounds in (RT)= 54.856

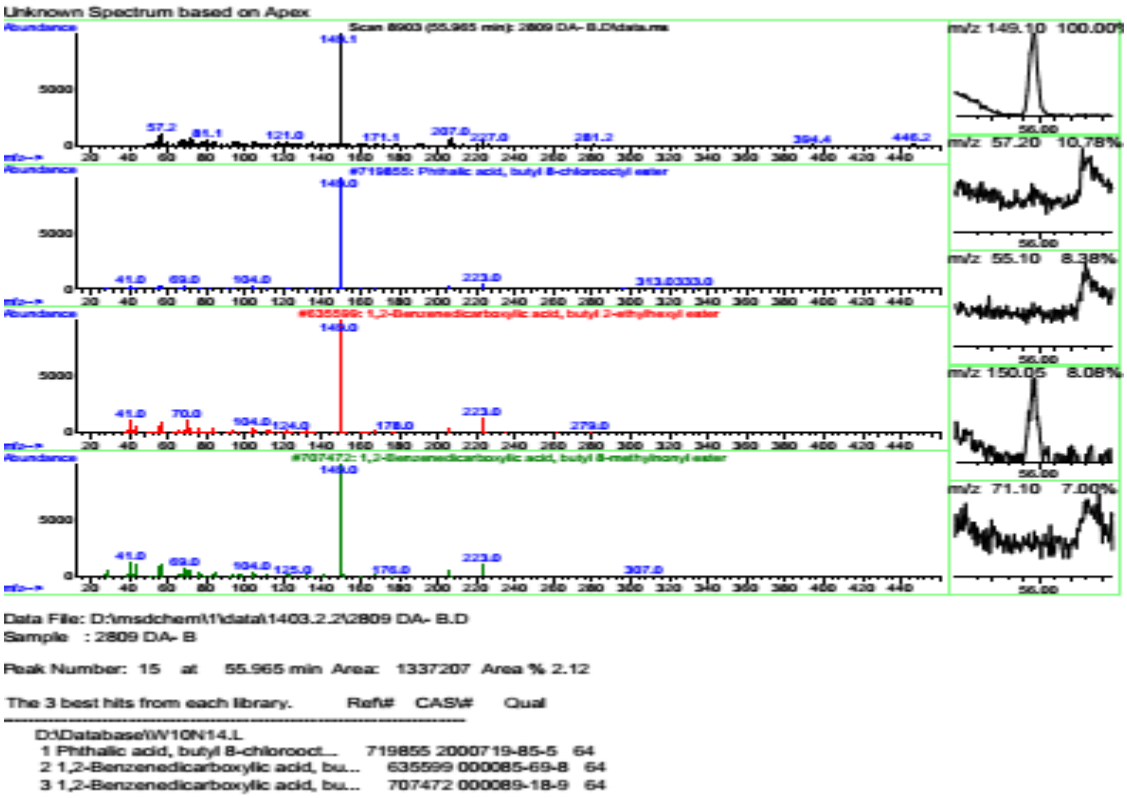


Figure 16. The revealed compounds in(RT)= 55.962

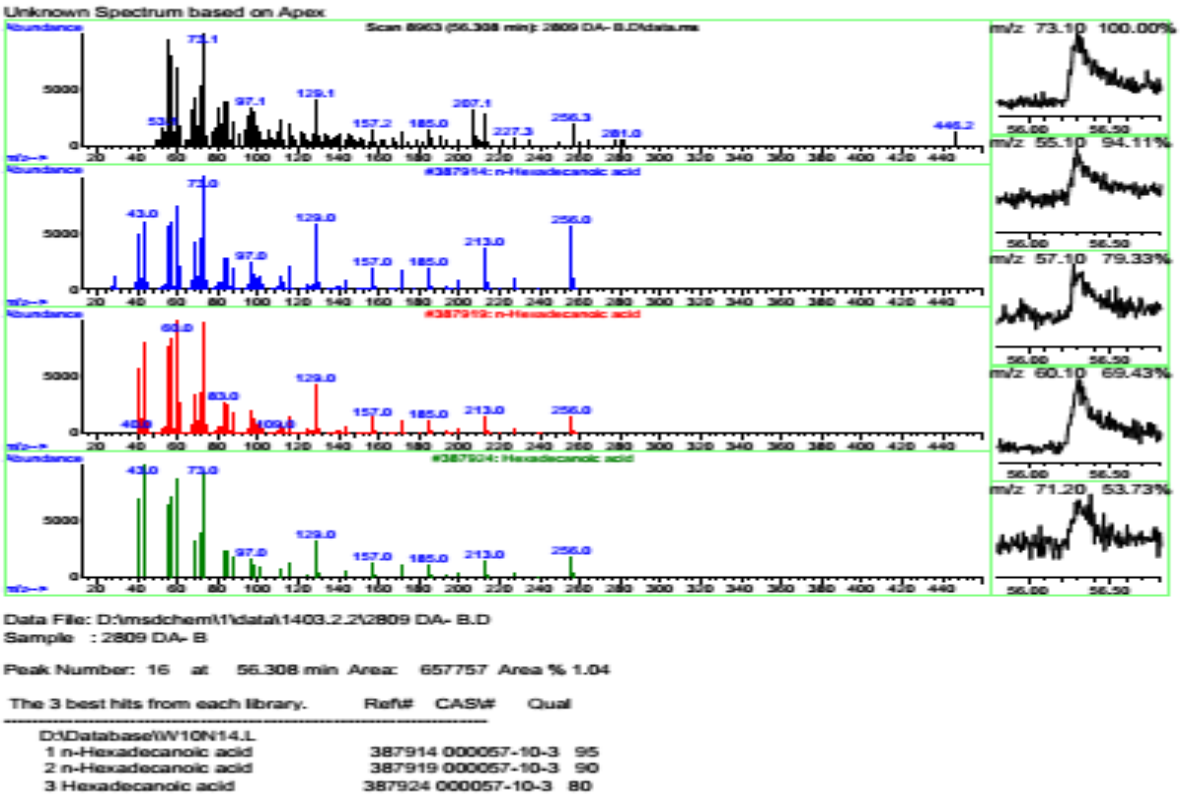
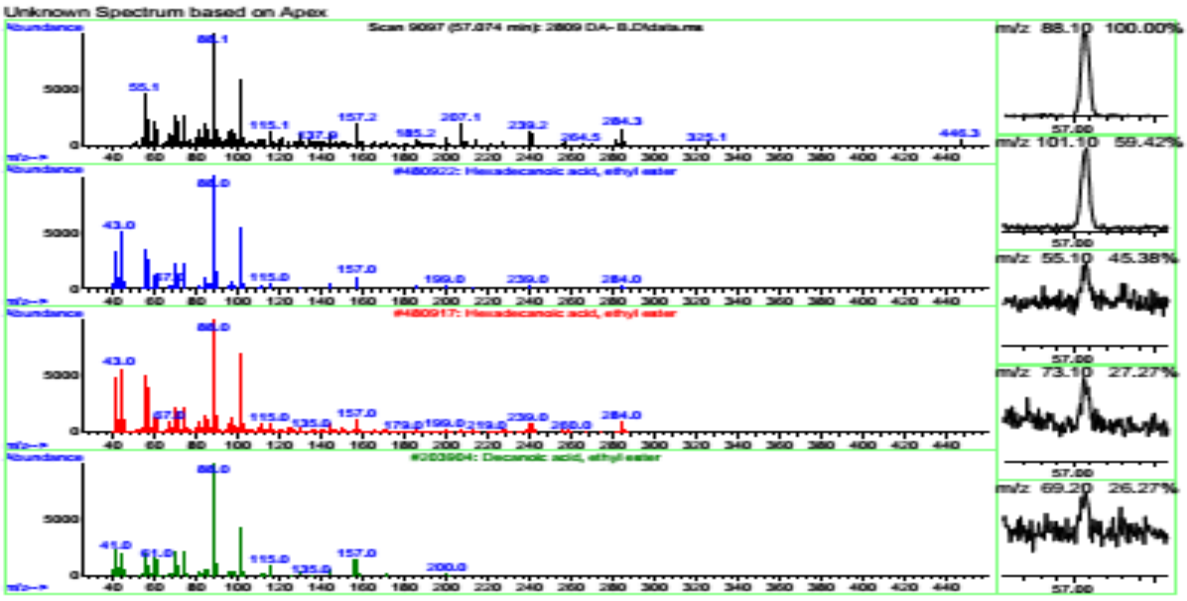


Figure 17. The revealed compounds in (RT)= 56.308

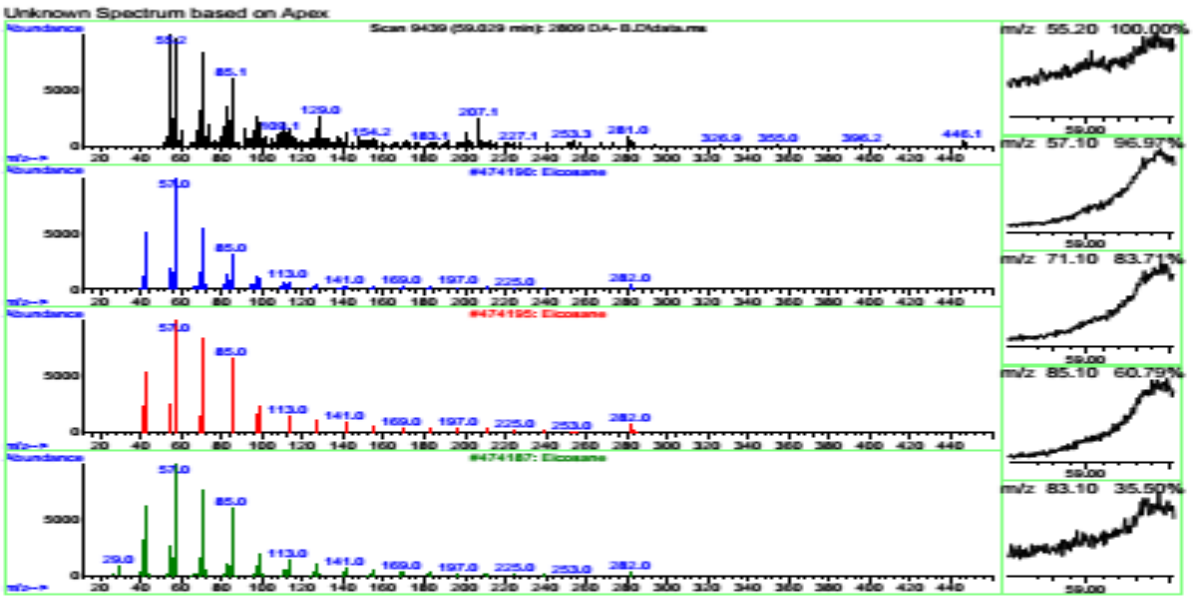


Data File: D:\msdchem\1\data\1403.2.2\2809 DA- B.D
Sample : 2809 DA- B

Peak Number: 17 at 57.074 min Area: 1595984 Area % 2.53

The 3 best hits from each library.	Ref#	CAS#	Qual
D:\Database\W10N14.L			
1 Hexadecanoic acid, ethyl ester	480922	000628-97-7	94
2 Hexadecanoic acid, ethyl ester	480917	000628-97-7	93
3 Decanoic acid, ethyl ester	203904	000110-38-3	93

Figure 18. The revealed compounds in (RT)= 51.074

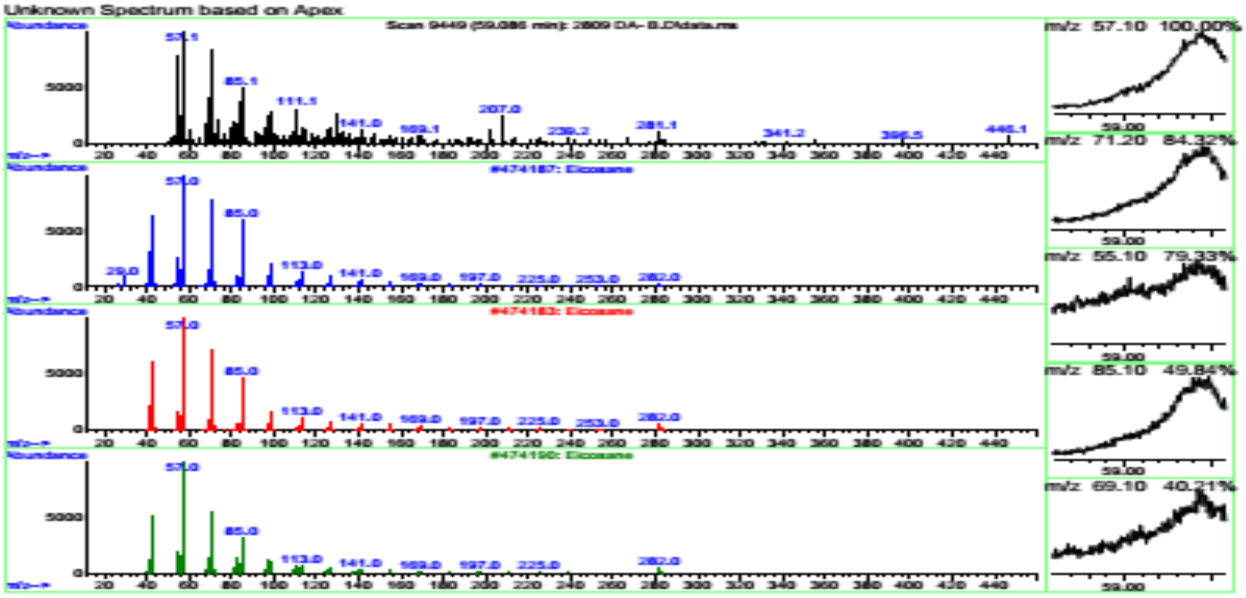


Data File: D:\msdchem\1\data\1403.2.2\2809 DA- B.D
Sample : 2809 DA- B

Peak Number: 18 at 59.029 min Area: 83611 Area % 0.13

The 3 best hits from each library.	Ref#	CAS#	Qual
D:\Database\W10N14.L			
1 Eicosane	474190	000112-95-8	95
2 Eicosane	474195	000112-95-8	92
3 Eicosane	474187	000112-95-8	91

Figure 19. The revealed compounds in (RT)= 59.029

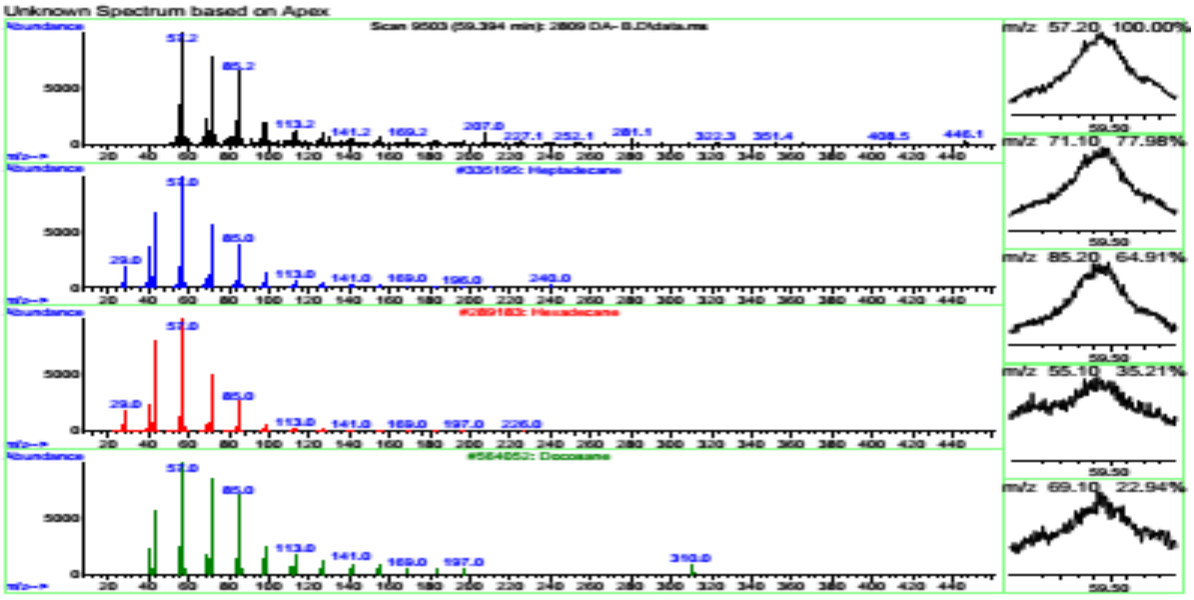


Data File: D:\msdchem\1\data\1403.2.2\2809 DA- B.D
Sample : 2809 DA- B

Peak Number: 19 at 59.086 min Area: 117402 Area % 0.19

The 3 best hits from each library.	Ref#	CAS#	Qual
D:\Database\W10N14.L			
1 Eicosane	474187	000112-95-8	90
2 Eicosane	474183	000112-95-8	70
3 Eicosane	474190	000112-95-8	64

Figure 20. The revealed compounds in (RT)= 59.086



Data File: D:\msdchem\1\data\1403.2.2\2809 DA- B.D
Sample : 2809 DA- B

Peak Number: 20 at 59.394 min Area: 4656947 Area % 7.37

The 3 best hits from each library.	Ref#	CAS#	Qual
D:\Database\W10N14.L			
1 Heptadecane	335195	000629-78-7	97
2 Hexadecane	289183	000544-76-3	93
3 Docosane	564052	000629-97-0	90

Figure 21. The revealed compounds in (RT)= 59.394

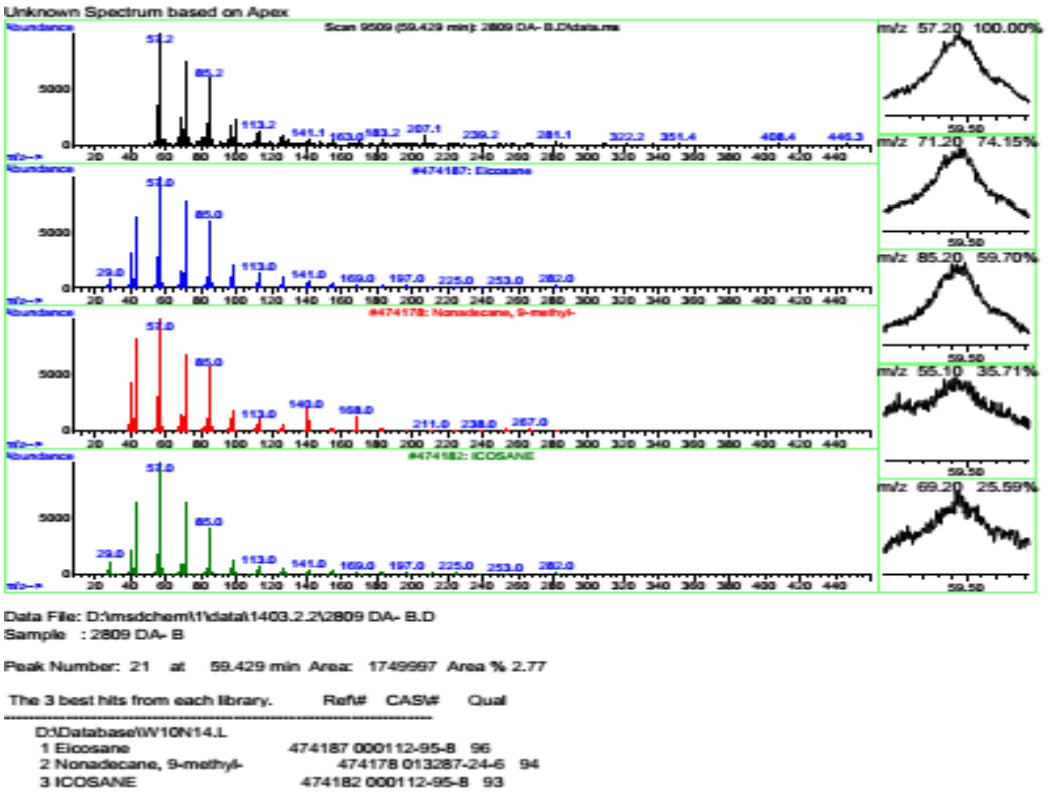


Figure 22. The revealed compounds in(RT)= 59.429

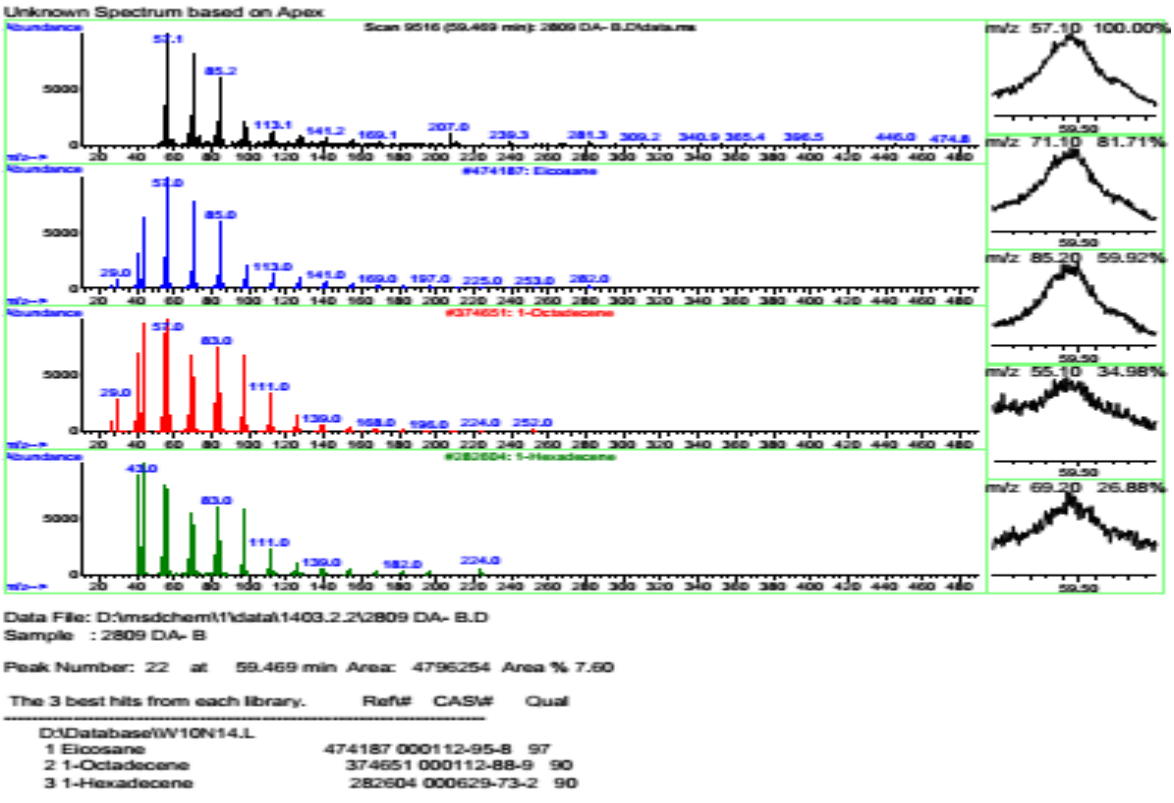


Figure 23. The revealed compounds in (RT)= 59.469

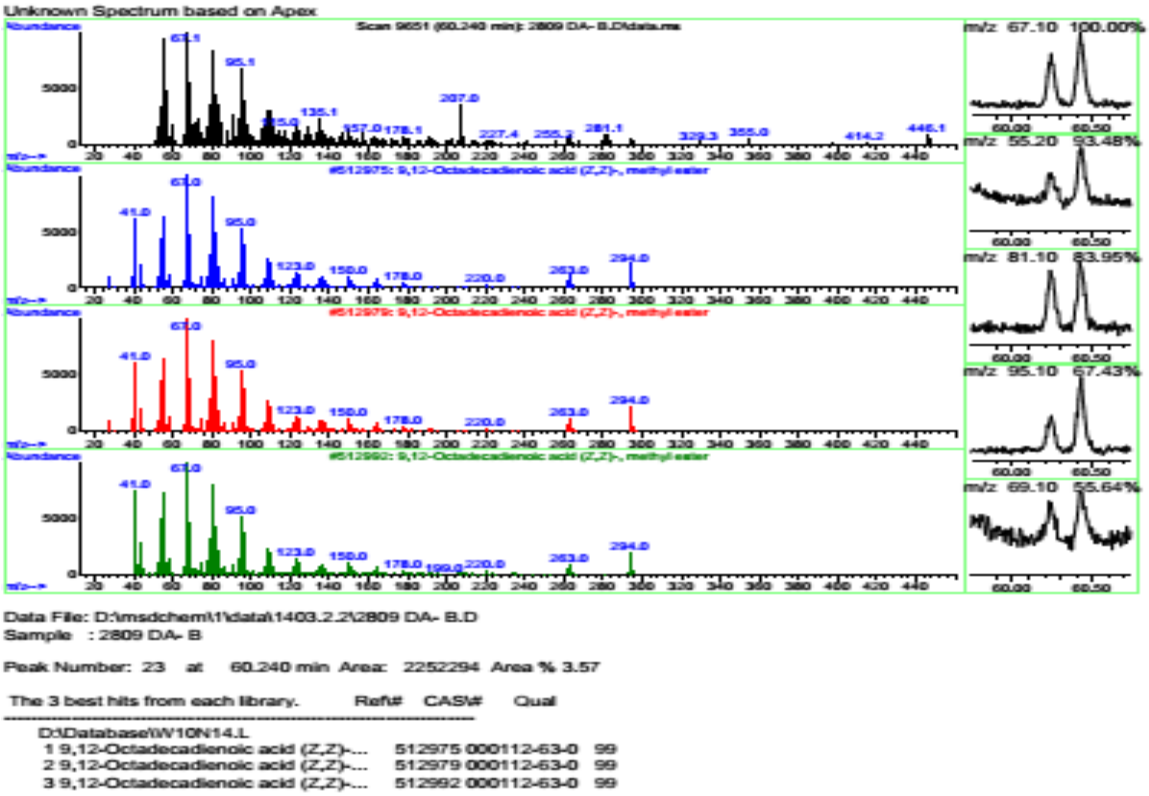


Figure 24. The revealed compounds in(RT)= 60.240

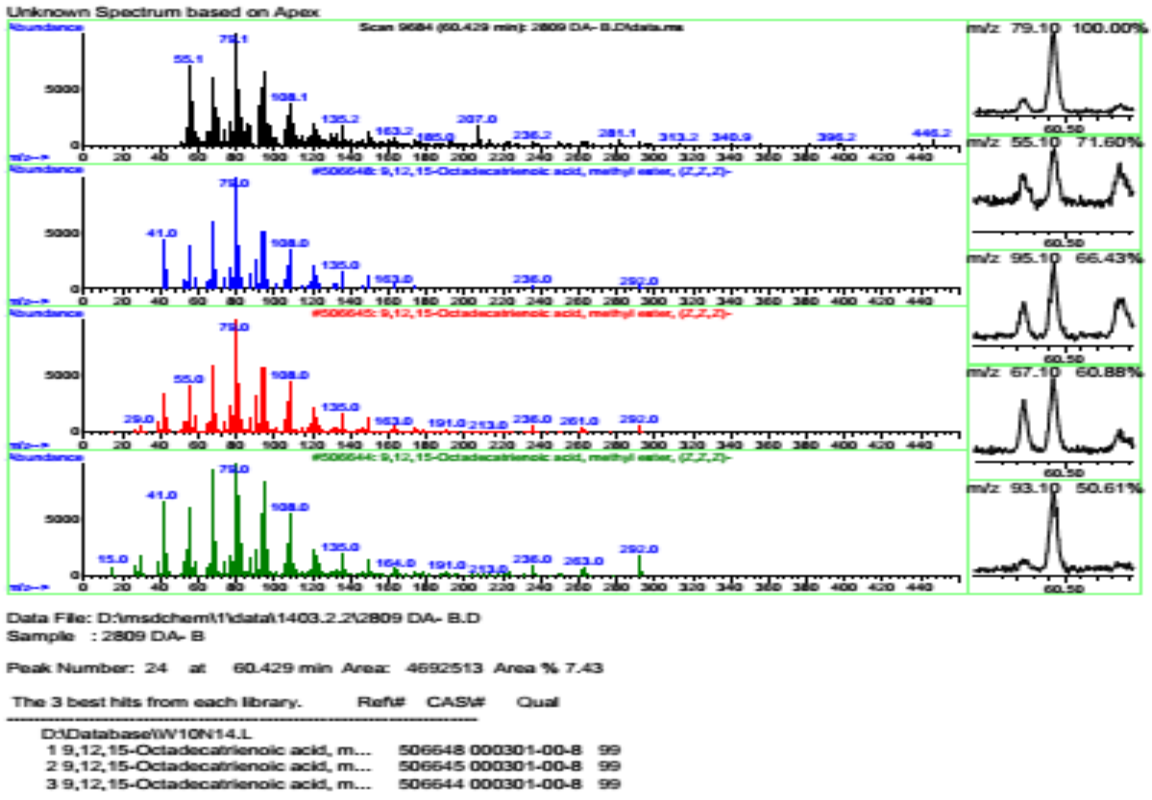


Figure 25. The revealed compounds in(RT)= 60.429

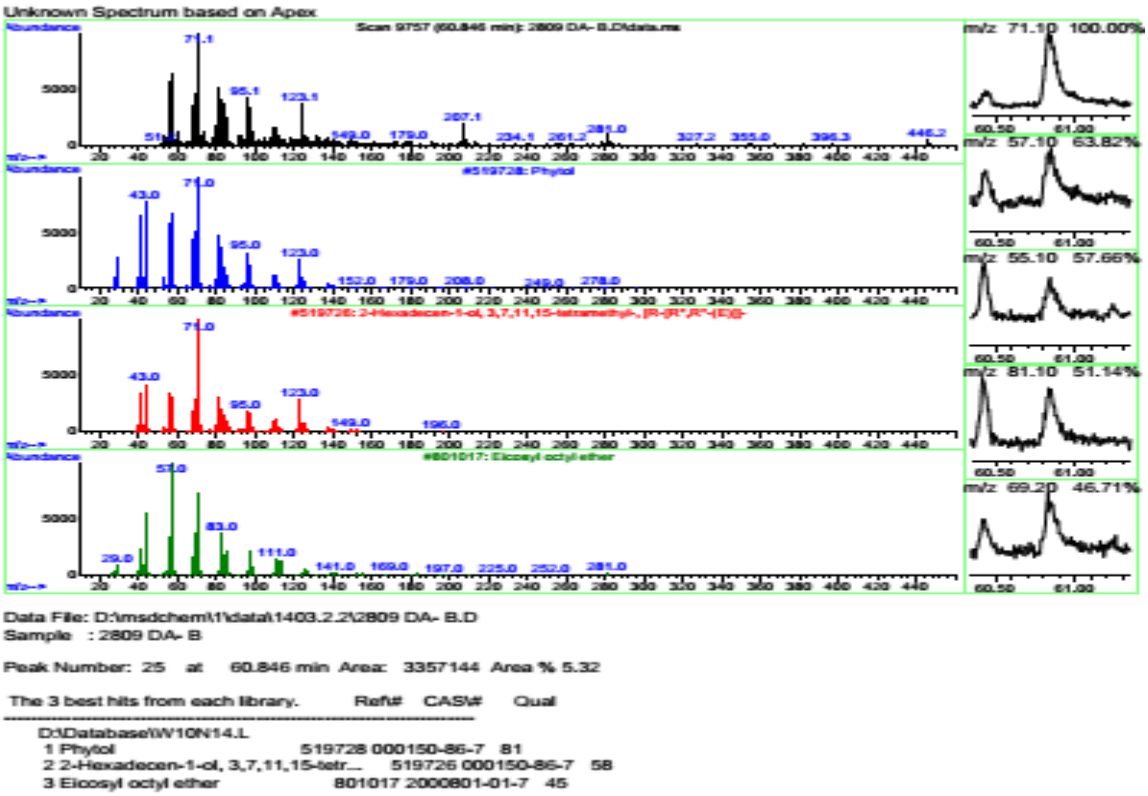


Figure 26. The revealed compounds in (RT)= 60.468

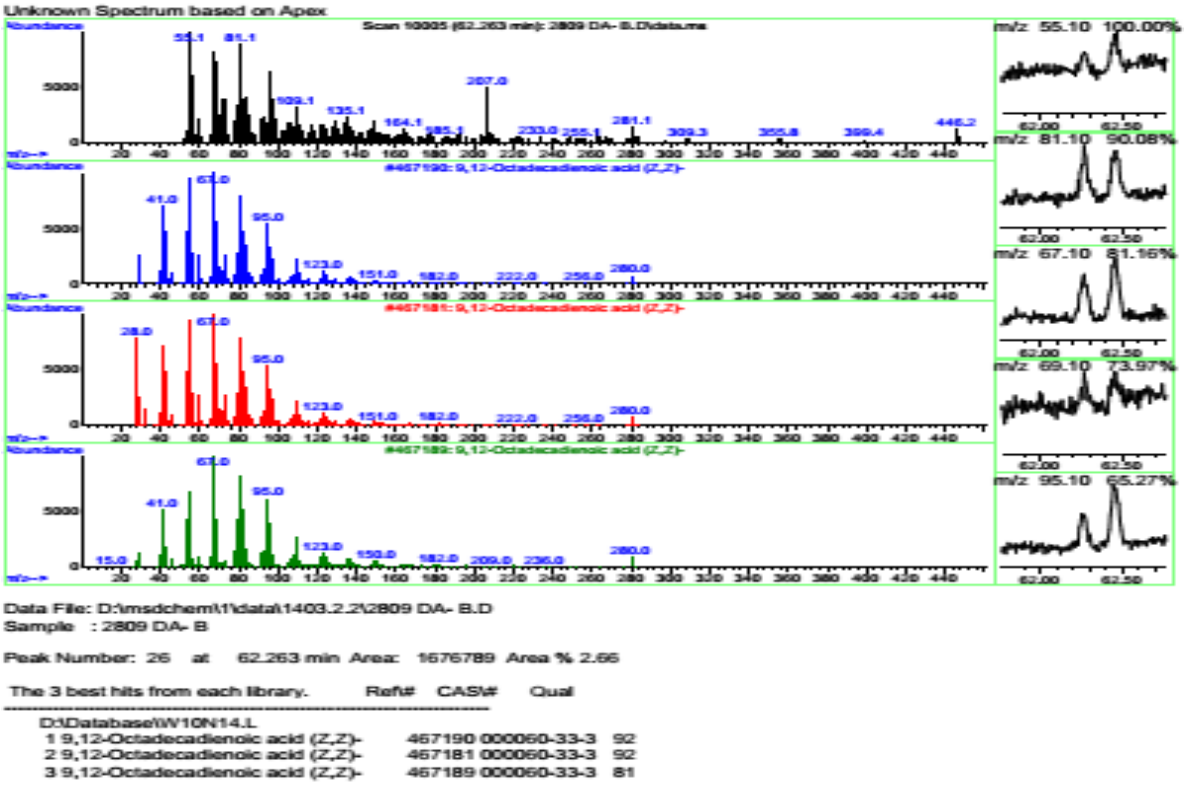


Figure 27. The revealed compounds in (RT)= 62.263

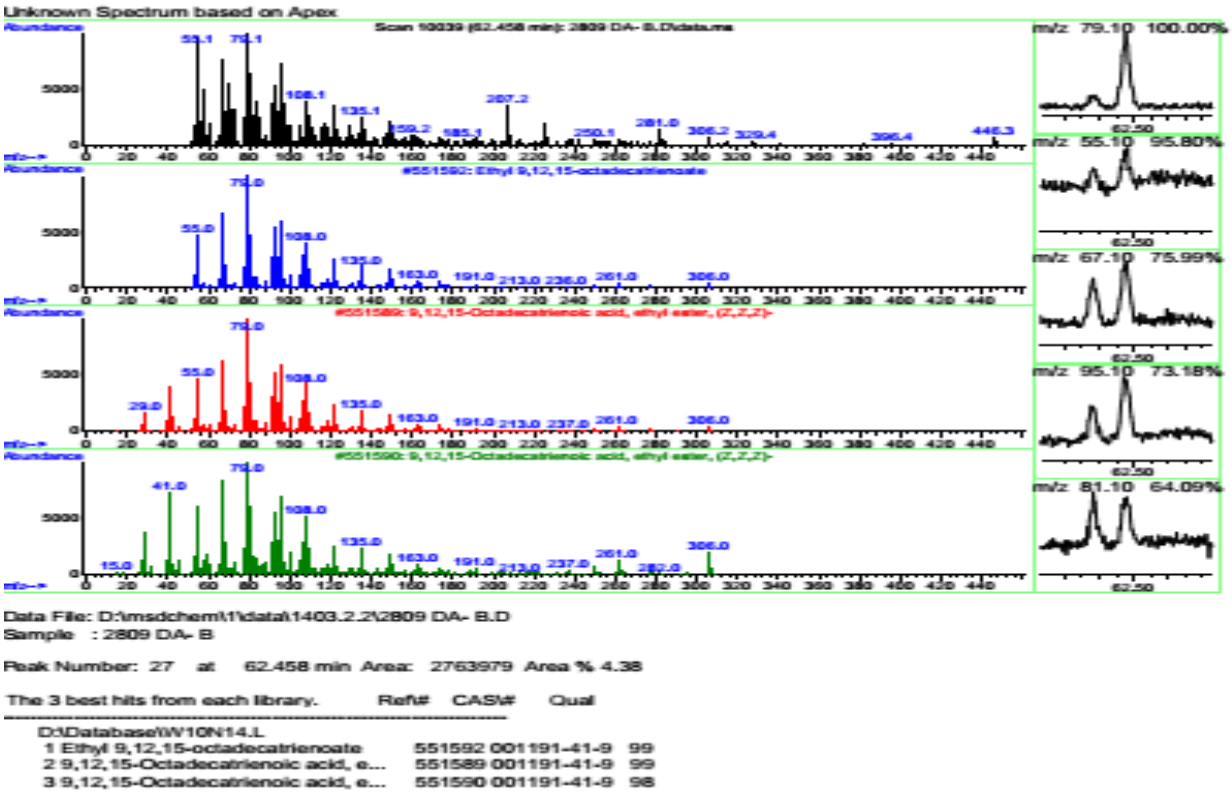


Figure 28. The revealed compounds by GC-MASS analysis in retention time (RT)= 62.458

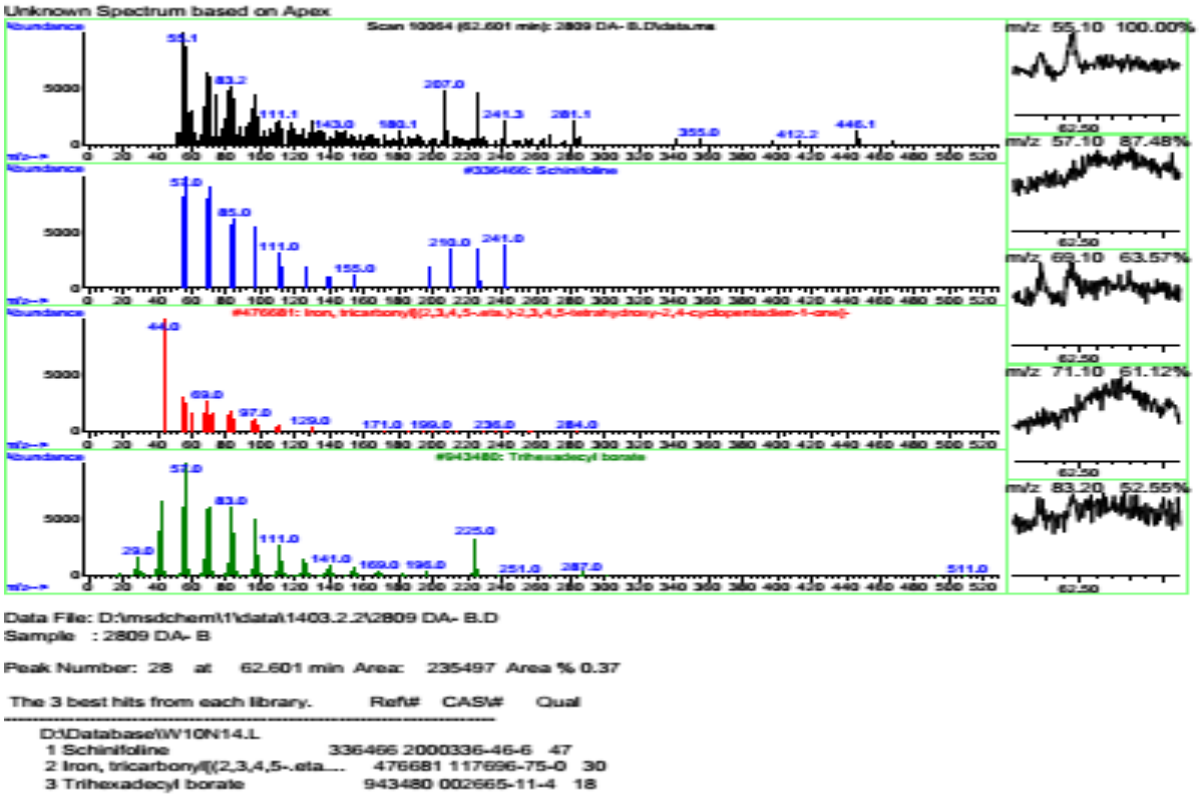


Figure 29. The revealed compounds in (RT)=62.601

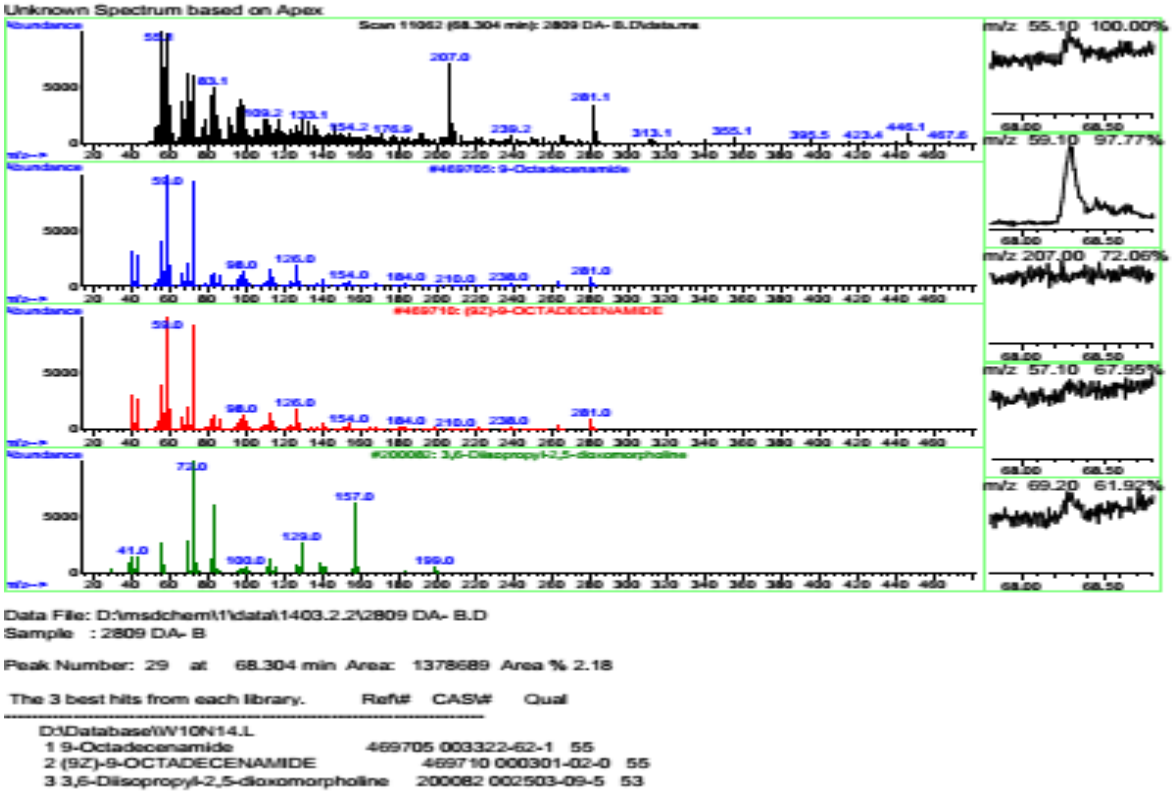


Figure 30. The revealed compounds in(RT)= 68.304

a. Antioxidant Activity

A DPPH radical scavenging experiment Results showed table 2 that the scavenging activity of *U. dioica* extract at even at 400 µg/ml concentrations is lower than of ascorbic acid reference antioxidant , but it is increasing with concentration,. These results indicate that extract is an effective antioxidant compared to ascorbic acid. This outcome agree with Bouaoudia-Madi *et al* (2017) that pointed out that efficient process of antioxidant activity depended on plant part localization as well as the extraction method. Fundamentally, the difference in antioxidant effectiveness may not be due to the amount of phenols but rather to their structures, according to the authors, nevertheless none study was performed by them to clarify this statement (Snoussi *et al*, 2021). Our finding be in harmony with Alkaltham *et al* (2021) that confirmed Myrtle fruit can be effectively used for the recovery of bioactive compounds which have play important role in functional qualities and may be utilized in the evolution of role food and nutrients. The hydrogen-donating hydroxyl groups quantity and placement of on the aromatic ring of phenolic compounds measured their capacity to radicals scavenge with work as antioxidants, but other secondary bioactive compounds, for example the glycosylation and groups of hydrogen donor, also have an impact (-NH and –SH). This outcome, may be generalized exam possessed of plant powerful antioxidant impact and capabilities free radical scavenging. This test made by referring to Ajewole and Adeyeye (1991).

Table 2. Calculated DPPH radical scavenging activity % of plant extracted at different concentration compared with ascorbic acid.

sample	Compounds Concentration µg/ml									
	0	40	80	160	320	400	600	800	1600	2400
U .dioica		22.4	35.32	42.7	53.62	76.26	93.76	95.95	95.96	96.10
Ascorbic acid		42.70	41.22	45.10	75.83	91.14	91.14	91.14	91.14	91.14

CONCLUSIONS

In short, *U. dioica* one of the many registered taxa providing substantial source of a phytochemicals compounds including phenolic compounds and terpenoids. Despite its used traditionally from different cultures to treat many diseeases , the present facts prove that *U. dioica* have renowned pharmacological scopes.

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