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A Chemical composition of Halophyte plant *Frankenia pulverulenta* L. (Frankeniaceae) in Iraq depending on GC-MS and FT-IR techniques

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ABSTRACT

The present study reported for the first time from Iraq about some detailed information on chemical composition of methanolic extract prepared from one *Frankenia* species namely *Frankenia pulverulenta* L. was investigated by Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier transform infrared (FT-IR) techniques. Results showed the presence of rich variety of phytochemical compounds approximately thirty bioactive components which may be responsible for pharmacological activities. Also, the study summarized the information concerning with various functional groups like alkenes, alkanes, aliphatic fluoro compounds, nitro compounds alcohols, ethers, carboxlic acids and esters were identified by FT-IR analysis compared with original and methanolic extract. At the same time making attention among the researchers in herbal analysis and this study creates an objective to screen many bioactive components to treat various diseases.

KEY WORDS: *Frankenia,* phytochemical compounds, methanolic extract, GC-MS and FT-IR.

1. INTRODUCTION

Frankenia L. is a global halophyte genus that come about in Mediterranean, semi-arid, and arid regions on special soil types which usually known as Sea Heath or MILLAIH belongs to the Frankeniaceae family (Whalen, 1987). It's the largest genus and considered only genus of the family that is local in the Western Hemisphere. In Iraq, *Frankenia* species have been systematically studying such as morphological, anatomical, palynological, ecological and geographical distribution have been done by (Al-Tameme, 2016), she was confirmed that presences two species of *Frankenia* namely *Frankenia pulverulenta* L. and *Frankenia aucheri*, also the first species was more commonly than the other (Figure.1).

Lewis (2003), pointed that none of *Frankenia* species have of economic or of exceptional ecological importance, but only research introduced by Fegler (1985), pointed to have beautiful flowers thus ornamental plant and some species contain gummy resin, kaempferol, quercetin and tannin (about 6%) utilized for sticking blade cutting edges and to seal stoneware.

Despite the spread of this species in many areas, particularly Iraq's neighboring regions such as Turkey (Webb, 1966), Lebanon, Jordan, and Palestine (Townsend, 1980), Egypt (Salama, 1999), Syria (Al-Oudat, 2011), Qatar (Abulfahij, 2002), Kuwait (Malallah, 2003) and Irano-Turanian and Mediterranean (Youcef, 2012) but no reports about phytochemicaly are available on this genus, therefore, the aim of this work was investigated bioactive components and determined by GC-MS and FT-IR techniques .

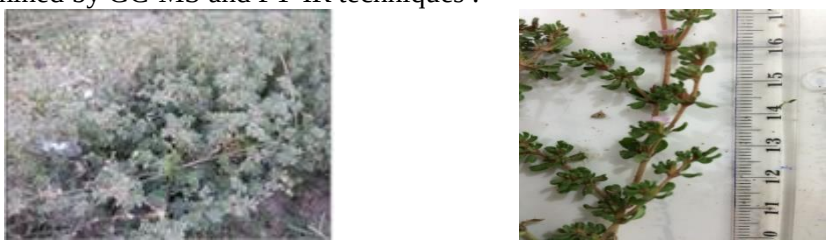

Figure.1. Habitat of of *Frankenia pulverulenta* and shape of leaves

2. MATERIALS AND METHODS

Collection plant and Preparation of Extraction: Dry leaves of *Frankenia pulverulenta* was collected randomly from various specimens were kept in Babylon university herbarium or directly from fields. Before use must make sure that the chosen leaves clean and free of dust or fungi. After that took the sample to crush by kitchen grinder for 20 second, finally the leaves sample were prepared as a powder form by using blender machine (Al-Tameme, 2015). According to Harborne (1984), A powdered (2-3) gm in each species was soaked and extracted for 48-72 hours with (20-30) ml of methanol at room temperature with shaker during the first six hours, Filtered the extracts by using filter paper (Whatman No.1) for removing the residue.

GC-MS Analysis and Identification of Components: 2µL of methanolic extract of leaves *F. pulverulenta* was performed on GC Clarus 500 Perkin Elmer interfaced to a mass spectrometer (GC-MS) and MS: Turbo mass gold Perkin Elmer Turbo mass 5.1 spectrometer and operating in EI mode at 70 eV, equipped with a split less injector

(250°C). The conditions were as follows: Column Elite-1ms fused silica capillary column (30×0.25mm ID x 0.25m film thickness, composed of 100% Dimethyl polysiloxane), Carrier gas Helium was at a continual flow of 1 ml/ min at 280ºC, and an injection volume of 2ml was used (10:1 split ratio), Temperature in ion-source was 200ºC. The temperature in oven was programmed from 40ºC, with an increment of 5ºCmin^{-1}, to 280ºC hold for 9 min. Mass spectra were taken at 70eV (electron ionization technique) at a scan interval of 0.5 seconds and fragments were scanned from 45 to 450 Da. Total running was 36min. Compounds in the chromatograms were determined by differentiation of their mass spectra with those in NIST library database, and by comparison of their retention index with those reported in the literature (Altameme, 2015).

FTIR Spectroscopic Analysis: 10mg of the dried extract powder was in flod in 100 mg of KBr pellet, in order to prepare gossamer sample disc. The powdered sample of each species was loaded in FT-IR Spectroscope (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4cm^{-1} (Jasim, 2015).

3. RESULTS AND DISCUSSION

Plants contain various bioactive chemical compounds, many of which are known to be biologically active constituents and are responsible for displaying different pharmacological activities (Gu, 2014). During the eighteenth and nineteenth centuries knowledge in the plant field increased and some taxonomists made use of several chemical characteristics in attempts to classify plants and to demonstrate their phylogeny (Al-Tameme, 2015). In the recent years, it has been demonstrated that Gas Chromatography coupled with Mass Spectrometry (GC-MS) is cleaner, faster and less expensive than the traditional extraction methods additionally it was precise method widely applied in diagnostics, functional genomics and for screening purposes. For these reason adopted this method in this work and the results showed a difference in the number of compounds in one species of the genus under study. Figures (2 and 33) showed the chromatogram obtained from the GC-MS of methanolic leaves extract of *F.pulverulenta* was displayed 30 peaks indicating the presence of thirty compounds. The list of constituents are given in Table.1. The prevailing components were Dihydrotecomanine; 5,7-Dodecadiyn-1,12-diol; 4H, 5H-Pyrano[4,3-d]-1,3-dioxin, tetrahydro-8a-methyl-;3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 3,6,9,12, -Tetraoxatetradecan-1-ol, 14-[4-(1,1,3,3-tetramethyl-; Dithiocarbamate, S-methyl-, N-(2-methyl-3-oxobutyl)-; 6-Acetyl-β-d-mannose; 1, 8-Di-(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one; Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester); Formamide, N-Methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-; N-Cyclooct-4-enylacetamide; 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one; 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester; 2-(2-Methyl-propenyl)-cyclohexanone oxime; 2-Oxabicyclo [3.3.0] oct-7-en-3-one, 7-(1-hydroxypentyl)-; 1-Propyl-3,6-diazahomoadamantan-9-ol; α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw-3)-β-; 3-O-Methyl-d-glucose; β-D-Glucopyranose , 4-O-β-D-galactopyranosyl-; n-Hexadecanoic acid; Curan, 16,17-didehydro-, (20xi)-; 1, 8-Diethyl-3,6-diazahomoadamantan-9-ol; Gamolenic acid; Curan-19,20-diol ,16,17-didehydro-, (19S)-; Gibberellic acid; Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-ly; Strychane, 1-acetyl-20α-hydroxy-16-methylene-; Dasycarpidan-1-methanol, acetate (ester); 2,7-Diphenyl-1,6-dioxopyradazino [4,5:2',3'] pyrrolo[4',5'-d] pyrid and Ethyl iso-allocholate (Figure 2-32).

The FTIR spectrum of crude and leaf extracts prepared in methanol are given in Figures 33 and 34. The data on the peak values and the probable functional groups present in tables 2 and 3. The absorption spectra of the of original powder of *F.pulverulenta* leaves are shown in table 2 and figure 33 exhibited a characteristic band at 788.89 and 871.81cm^{-1} indicating the presence of C-H stretching and the peaks on 1008.77 and 1039.63 cm^{-1} are corresponded to C-F stretching as Aliphatic fluoro compounds, and 1190.08 cm^{-1} for C-O group, the beak area at 1398.39cm^{-1} shows symmetric NO_2 stretching, furthermore unknown compound at the peak is appeared in 1606.70 cm^{-1} compare with the compounds in methanolic extract *F. pulverulenta* indicated shows that the band at 1012.63, 1099.43 and 1226.73cm^{-1} attributed to C-F and C-O stretching (Table.3 and Figure.34).

So the outcome of this study united in opinion with the past observations which found by numerous plant biologist and taxonomist. The results of the spectrometry have confirmed the presence of effective constituents in the crude powder and methanolic extracts of *Frankenia* species which are responsible for many biological activities such as the study from Wided (2011), when they were proved having *Frankeni athymifolia* Desf. antioxidant and antimicrobial properties. While Abdel-Hamid (2014) study, which confirmed presence Octadecanoic acid and n-Hexadecanoic acid compounds in halophyte plant could be the useful candidates to serve as a genetic source for this reason. Also, the presence of Ethyl iso-allocholate compound was made having Anti-inflammatory activity and a compound that namely Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester) has antiviral and antitumor activity (Hussein, 2016), α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1. fwdarw-3)-β-has antidiabetic activity and antitumour, and Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en- 17-yl) has antimicrobial and antitumor effects (Sosa, 2016). Thus many researchers considered the GC-MS and FT-IR analysis as a device for characteristic

closely related plants and other organisms (Liu, 2006; Duraes, 2008). Thus in future we need a next progressive spectroscopic studies are required for the structural illustration and recognition of bioactive principles present in the leaves *F.pulverulenta* could be used in practical research in medicine, agriculture and industry.

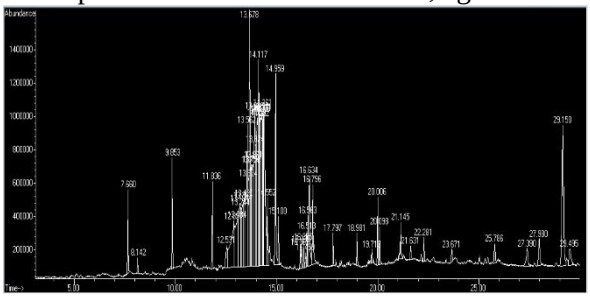

Figure.2. Chromatogram obtained from the GC-MS of *F.pulverulenta*

Table.1. Components detected in extract of *F.pulverulenta*leaves

Name of the compound	Retention time (min)	Exact mass	Molecular formula	Structure
Dihydrotecomanine	3.396	181.146665	$C_{11}H_{19}NO$	Figure 3
5,7-Dodecadiyn-1,12-diol	3.968	194.13068	$C_{12}H_{18}O_2$	Figure 4
4H,5H-Pyrano[4,3-d]-1,3-dioxin, tetrahydro-8a-methyl-	4.380	158.094295	$C_8H_{14}O_3$	Figure 5
3-tert-Butyl-5-chloro-2-hydroxybenzophenone	4.849	288.091707	$C_{17}H_{17}ClO_2$	Figure 6
3,6,9,12, -Tetraoxatetradecan-1-ol, 14-[4-(1,1,3,3-tetramethyl-	5.313	426.29814	$C_{10}H_{22}O_4$	Figure 7
Dithiocarbamate, S-methyl-, N-(2-methyl-3-oxobutyl)-	5.753	191.043856	$C_7H_{13}NOS_2$	Figure 8
6-Acetyl-β-d-mannose	6.028	222.073953	$C_8H_{14}O_7$	Figure 9
1, 8-Di-(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one	7.178	436.17467	$C_{23}H_{24}N_4O_5$	Figure 10
Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	8.906	197.068808	$C_9H_{11}NO_4$	Figure 11
Formamide, N-Methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-	9.072	180.126264	$C_{10}H_{16}N_2O$	Figure 12
N-Cyclooct-4-enylacetamide	10.440	167.131014	$C_{10}H_{17}NO$	Figure 13
12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	10.794	240.1725445	$C_{14}H_{24}O_3$	Figure 14
11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	11.235	270.18311	$C_{15}H_{26}O_4$	Figure 15
2-(2-Methyl-propenyl)-cyclohexanone oxime	11.429	167.131014	$C_{10}H_{17}NO$	Figure 16
2-Oxabicyclo [3.3.0] oct-7-en-3-one, 7-(1-hydroxypentyl)-	12.059	210.125594	$C_{12}H_{18}O_3$	Figure 17
1-Propyl-3,6-diazahomoadamantan-9-ol	12.078	210.173213	$C_{12}H_{22}N_2O$	Figure 18
α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1. Fwdarw-3)-β-	12.580	504.169035	$C_{18}H_{32}O_{16}$	Figure 19
3-O-Methyl-d-glucose	13.180	194.079039	$C_7H_{14}O_6$	Figure 20
β-D-Glucopyranose , 4-O-β-D-galactopyranosyl-	14.634	342.11621	$C_{12}H_{22}O_{11}$	Figure 21
n-Hexadecanoic acid	14.966	256.24023	$C_{16}H_{32}O_2$	Figure 22
Curan, 16,17-didehydro-, (20xi)-	15.950	280.193949	$C_{19}H_{24}N2$	Figure 23
1, 8-Diethyl-3,6-diazahomoadamantan-9-ol	16.144	224.188864	$C_{13}H_{24}N_2O$	Figure 24
Gamolenic acid	16.722	278.22458	$C_{18}H_{30}O_2$	Figure 25
Curan-19,20-diol ,16,17-didehydro-, (19S)-	17.077	312.183779	$C_{19}H_{24}N_2O_2$	Figure 26
Gibberellic acid	17.489	346.141638	$C_{19}H_{22}O_6$	Figure 27
Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-ly	17.815	430.30831	$C_{27}H_{42}O_4$	Figure 28
Strychane, 1-acetyl-20α-hydroxy-16-methylene-	17.924	338.199429	$C_{21}H_{26}N_2O_2$	Figure 29

Dasycarpidan-1-methanol, acetate (ester)	18.588	326.199429	$C_{20}H_{26}N_2O_2$	Figure 30
2,7-Diphenyl-1,6-dioxopyradazino [4,5:2',3'] pyrrolo[4',5'-d] pyrid	18.914	355.106924		Figure 31
Ethyl iso-allocholate	21.191	436.318874	$C_{26}H_{44}O_5$	Figure 32

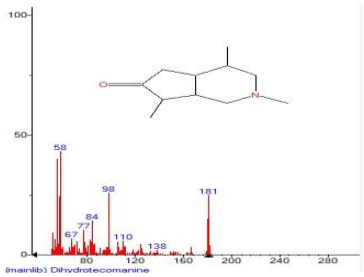

Figure.3. Chromatogram of Dihydrotecomanineinein *F.pulverulenta*

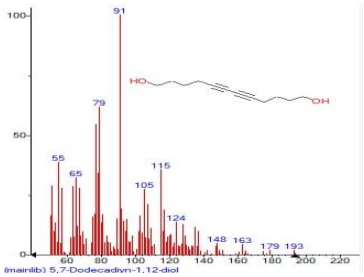

Figure.4. Chromatogram of 5,7-Dodecadiyn-1,12-diol in *F.pulverulenta*

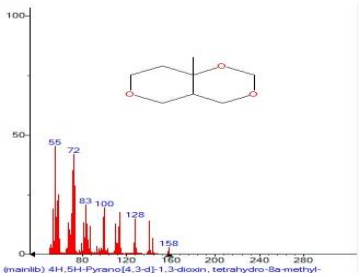

Figure.5. Chromatogram of 4H,5H-Pyrano[4,3-d]-1,3-dioxin, tetrahydro-8a-methyl- in *F.pulverulenta*

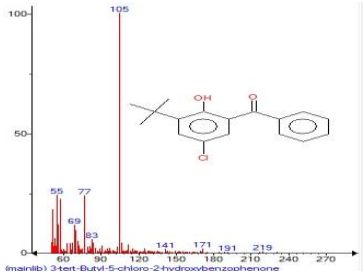

Figure.6. Chromatogram of 3-tert-Butyl-5-chloro-2-hydroxybenzophenone in *F.pulverulenta*

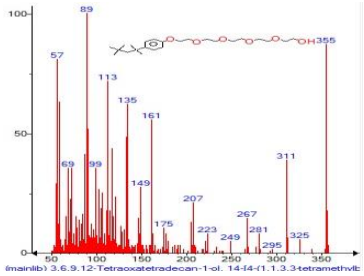

Figure.7. Chromatogram of 3,6,9,12, -Tetraoxatetradecan-1-ol, 14- [4-(1,1,3,3-tetramethyl- in *F.pulverulenta*

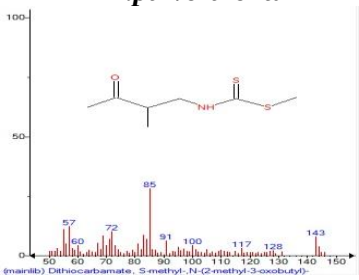

Figure.8. Chromatogram of Dithiocarbamate, S-methyl-, N-(2-methyl-3-oxobutyl) in *F.pulverulenta*

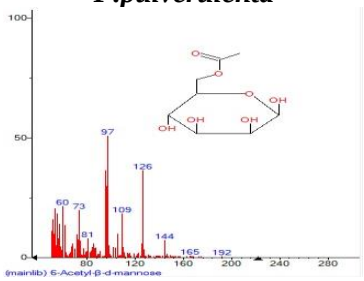

Figure.9. Chromatogram of 6-Acetyl-β-d-mannose in *F.pulverulenta*

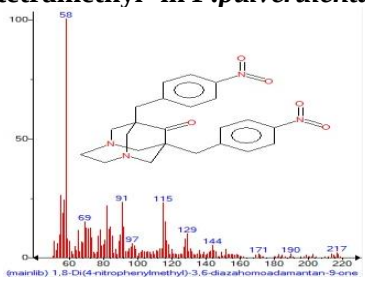

Figure.10. Chromatogram of 1, 8-Di-(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one in *F.pulverulenta*

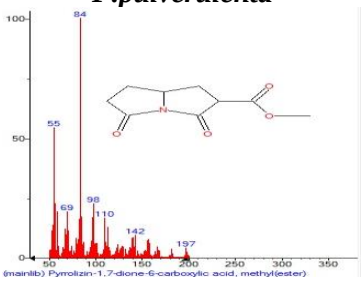

Figure.11. Chromatogram of Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester) in *F.pulverulenta*

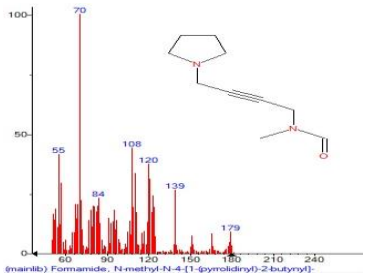

Figure.12. Chromatogram of Formamide,N-Methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]- in *F. pulverulenta*.

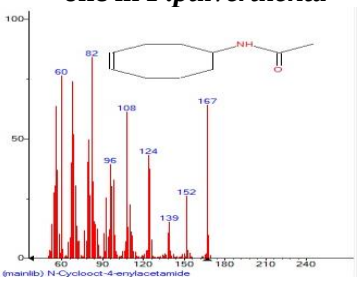

Figure.13. Chromatogram of N-Cyclooct-4-enylacetamide in*F. pulverulenta*.

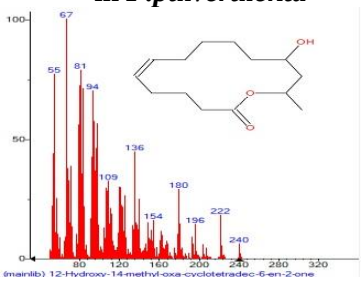

Figure.14. Chromatogram of 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one in *F. pulverulenta*.

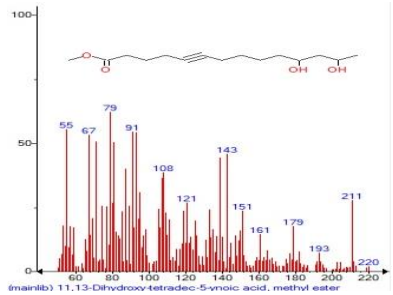

Figure.15. Chromatogram of 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester in*F.pulverulenta*

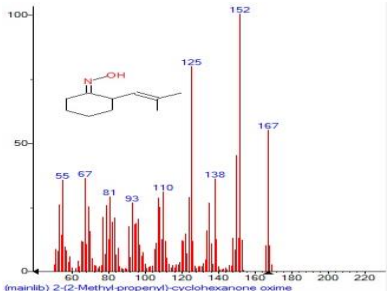

Figure.16. Chromatogram of 2-(2-Methyl-propenyl)-cyclohexanone oxime in *F.pulverulenta*

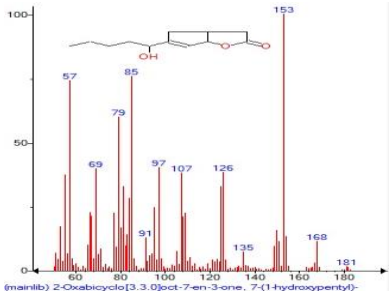

Figure.17. Chromatogram of 2-Oxabicyclo [3.3.0] oct-7-en-3-one, 7-(1-hydroxypentyl)- in *F.pulverulenta*

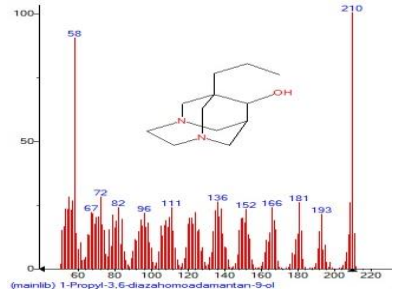

Figure.18. Chromatogram of 1-Propyl-3, 6-diazahomoadamantan-9-ol in *F.pulverulenta*

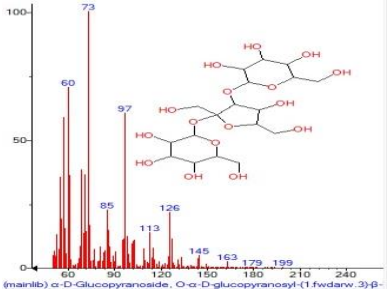

Figure.19. Chromatogram of α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1. fwdarw-3)-β- in *F.pulverulenta*

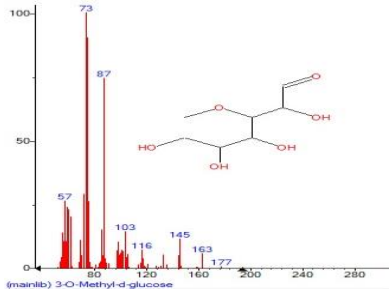

Figure.20. Structure 3-O-Methyl-d-glucose in *F.pulverulenta*

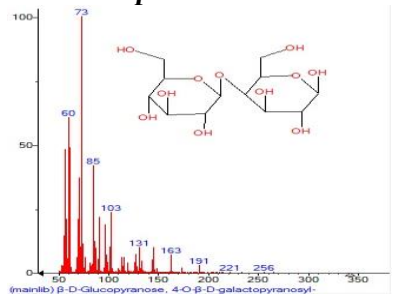

Figure.21. Chromatogram of β-D-Glucopyranose , 4-O-β-D-galactopyranosyl- in *F.pulverulenta*

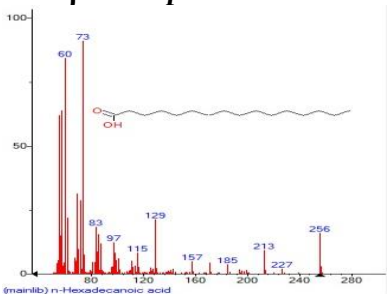

Figure.22. Chromatogram of n-Hexadecanoic acid in *F.pulverulenta*

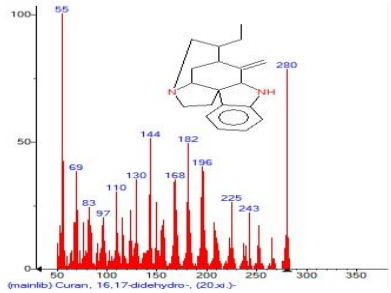

Figure.23. Chromatogram of Curan, 16,17-didehydro-, (20xi)- in *F.pulverulenta*

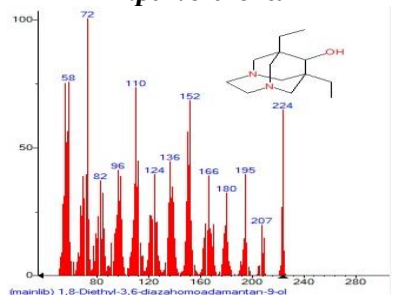

Figure.24. Chromatogram of 1, 8-Diethyl-3,6-diazahomoadamantan-9-ol in *F.pulverulenta*.

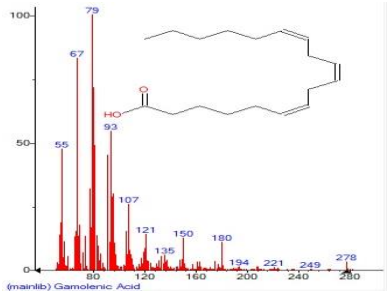

Figure.25. Chromatogram of Gamolenic acid in *F.pulverulenta*.

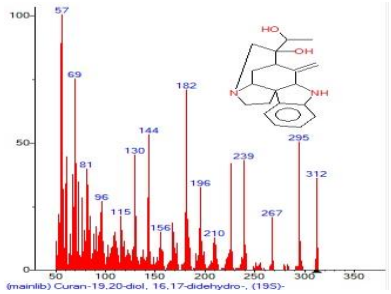

Figure.26. Chromatogram of Curan-19,20-diol ,16,17-didehydro-, (19S)- in *F.pulverulenta*.

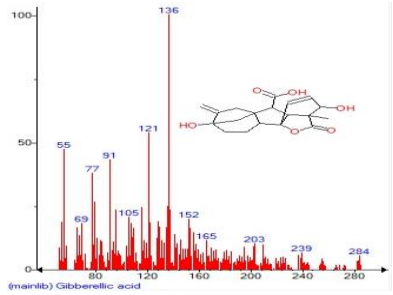

Figure.27. Chromatogram of Gibberellic acid in *F.pulverulenta*

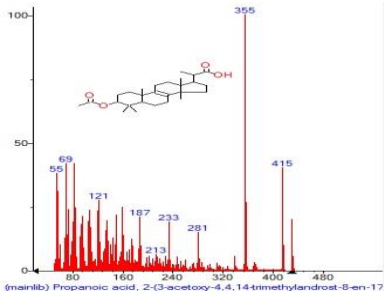

Figure.28. Chromatogram of Propanoicacid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-lyin *F.pulverulenta*

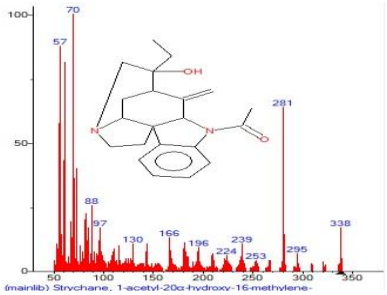

Figure.29. Chromatogram of Strychane, 1-acetyl-20α-hydroxy-16-methylene- in *F.pulverulenta*

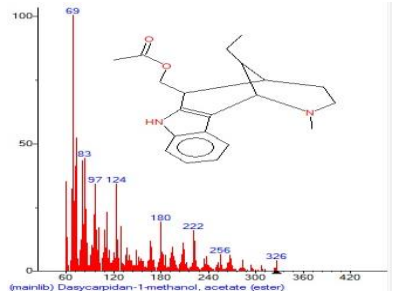

Figure.30. Chromatogram of Dasycarpidan-1-methanol, acetate (ester) in *F. pulverulenta*

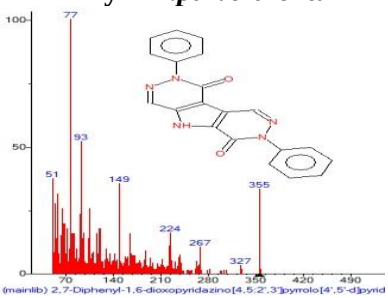

Figure.31. Chromatogram of 2,7-Diphenyl-1,6-dioxopyradazino [4,5:2',3'] pyrrolo [4',5'-d] pyridin *F.pulverulenta*.

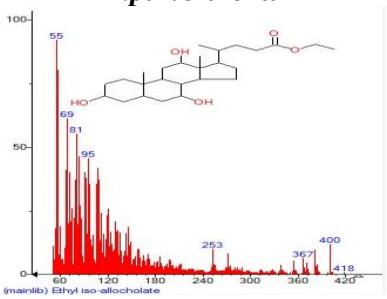

Figure.32. Chromatogram of Ethyl iso – allocholatein *F. pulverulenta.*

Table.2. FT-IR peak values of solid analysis of *F. pulverulenta* leaves (original).

Peak values (Wave number cm-^{l})	Intensity	Stretching	Functional group	Group frequency
788.89	76.618	C-H	Alkenes	675-995
871.82	75.371	C-H	Alkenes	675-995
1008.77	63.523	C-F	Aliphatic fluoro compounds	1000-1050
1039.63	64.501	C-F	Aliphatic fluoro compounds	1000-1050
1190.08	78.517	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
1398.39	77.011	NO2	Nitro Compounds	1300-1370
1606.70	78.663	-	Unknown	-

Table.3. FT-IR peak values of solid analysis methanolic extract of *F. pulverulenta* leaves

Peak values (Wave number cm-^{l})	Intensity	Stretching	Functional group	Group frequency
1012.63	72.415	C-F	Aliphatic fluoro compounds	1000-1050
1099.43	77.399	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
1226.73	84.800	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300

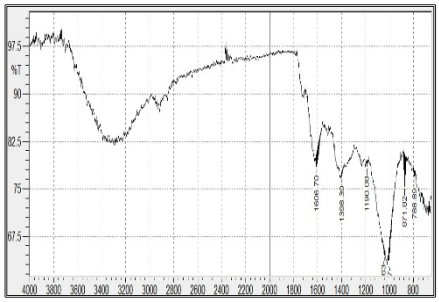

Figure.33. FT-IR profile solid analysis of *F. pulverulenta* leaves (original)

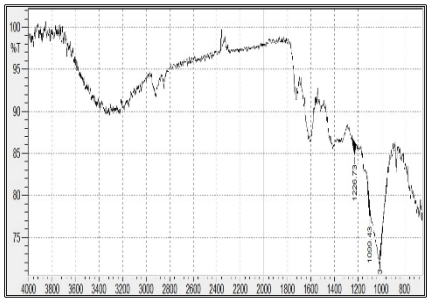

Figure.34. FT-IR profile solid analysis of *F. pulverulenta* leaves methanolic extract

4. CONCLUSION

Phytochemical screening of *Frankenia pulverulenta* carried by GC-MS and FT-IR spectral analysis techniques have led to the isolation and identification of a number of structurally diverse chemical compounds and presence of various functional groups in crude and methanolic extract which are responsible for many biological activities. Hence this kind of spectral investigations is the primary move towards acknowledgment the nature of active principles in this medicinal plant which will be useful for further detailed study.

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