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Soxhtherm Extraction, Isolation and Identification of Fatty Acids Present in the Hexane Extract of Abutilon Pannosum and Grewia Tenax Using Gas Chromatography-Mass Spectrometry

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Abstract: *Plants have been significant source of drugs with potential for thousands of years. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. [1] GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. [2] The main objective of the study was to evaluate the fatty acids composition for identified bioactive compound in leaf hexane extract of the Abutilon pannosum and Grewia tenax was analysed by gas chromatography combined with Mass Spectrometry. While the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Fatty acids play a crucial role in growth and development of the body. It is known to have antibacterial and antifungal properties. [3]The shade dried both plant leaf powder was extracted with hexane by using Gerhardt Soxhtherm extractor and crude hexane extract was obtained. Derivatization was performed and then gas chromatography-mass spectrometry (GC-MS) was done for detecting fatty acids. From that eleven phytochemical constituents have been identified. The GC-MS analysis revealed the presence of various compounds like 9, 12-Octadecadienoic, 9-Octadecenoic acid, Hexadecanoic acid, Octadecanoic acid, Octadecatrienoic acid, 9, 12, 15- Eicosanoic acid, 9-Hexadecenoic acid, 11-Eicosenoic acid, Methyl tetradecanoate, Octanoic acid and Tridecanoic acid. These findings support the traditional use of A. pannosum and G. tenax in various disorders.*

Keywords: *Fatty acid, GC-MS, Abutilon pannosum and Grewia tenax*

1. INTRODUCTION

Abutilon pannosum and *Grewia tenax* is an important medicinal plant in the Indian system of Medicine. It is commonly called khapat or kanghi and gangeti or gudaim, which grows in warm and arid regions. *A. pannosum* is used in cleaning wound and ulcer, treating vaginal infection, diabetics, haemorrhoids and can also use as an anaemia. [4]*G. tenax* is used tonsillitis, bone fracture and swelling, lactation, anaemia, porridge. [5] Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra. [6] Fatty acids are widely occurring in natural fats and dietary oils, and they are also important nutritious substances and metabolites in living organisms. [7] The human body needs essential fatty acids to construct and repair cell membranes enabling the cells to obtain optimum nutrition and expel harmful waste products.[8]A primary function of essential fatty acids, which support the cardiovascular, reproductive, immune and nervous systems, is the production of prostaglandins. [9]These regulate body functions such as heart rate, blood pressure, blood clotting, fertility and play a role in immune system by regulating inflammation. [10-12] Yet there is no report about the fatty acid composition of hexane extract leaves of *A. pannosum* and *G. tenax* species, which is the subject of the present study.

2. MATERIALS AND METHODS

2.1. Extraction Method

About 20 g of powdered material of aerial parts of two selected plants (*A. pannosum* and *G. tenax*) were extracted with 750 mL n-hexane for six hours through Gerhardt soxtherm apparatus. The

extracts were concentrated by recovering the solvent using rotary evaporator. The next step was derivatization of the fatty acids in order to make them volatile to be capable of being analysed with gas chromatography-mass spectrometry (GC-MS). Methylation is the most general method of converting non-volatile fatty acids into volatile fatty acids methyl esters.

2.2. Derivatization Method

First 100 mg of extract was weighed in 250 ml Round bottom flask and kept it on a heating mantle at 45^{o}C. After that 4 ml methanolic NaOH was added and boiled for 5 minutes. Next 2 ml Boron tri-fluoride was added and boiled it for 5 minutes. Then heating was stopped and 4 ml Hexane was added and round bottom flask (r.b.f.) was removed from the heating mantle and 15 ml saturated Nacl was added allow r.b.f. to cool and mixture was transferred to a test tube for phase separation. After that 0.5-1 gm sodium sulphate was taken in the eppendrof and the upper phase was transferred into it and vortex for 2 minutes for moisture and water removal and last 1.5 ml of oil is transferred into GC vial. **[13]**

2.3. GC-MS Method

The plant extract samples were analysed using Shimadzu GC-2010 system comprising an AOC-20i auto-sampler and interfaced to a Mass Spectrometer (QP Plus 2010) equipped with a DB-wax (100% Poly-Ethylene Glycol, polar fused capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in Electron Impact (EI) mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed (a split ratio of 50:1). The injector temperature was maintained at 250°C, the ion-source temperature was 230°C, the oven temperature was programmed from 60°C with an increase of 12°C/min to 150°C (isothermal for 1 min), then the oven temperature was increased at a rate of 5°C/min to 240°C (isothermal for 5 min), Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 50 m/z to 1000 m/z. The solvent delay was 0 to 2.30 min, and the total GC/MS running time was 31.50 min.

2.4. Identification Method

Identification of the fatty acid methyl ester was conducted by comparing the mass spectrum with NIST library. The compounds showing more that 90% Similarity Index (SI) was identified and recorded further characterization. The bioactivities of the identified compounds related to medicinal and chemical property were identified from online database of NIST, PubChem, PubMed and Chem Spider etc.

3. RESULT AND DISCUSSION

GC–MS chromatogram of the hexane extract of leaves of *Abutilon pannosum* (Fig. 1) clearly showed 11 peaks and G. tenax has showed 9 peaks that were indicating the presence of 11 and 9 phytochemical compounds respectively. The identification of the phytochemical compounds was founded on the peak area, retention time and molecular formula. The table 1 shows the compound name with its molecular formula, Retention time, Peak area and % Peak area. The results reveal the presence of 9, 12-Octadecadienoic (45.05%), 9-Octadecenoic acid (33.18%), Hexadecanoic acid (12.12%), Octadecanoic acid (5.88%), Octadecatrienoic acid (2.62%), 9, 12, 15- Eicosanoic acid (0.60%), 9-Hexadecenoic acid (0.22%), 11-Eicosenoic acid (0.16%), Methyl tetradecanoate (0.10%), Octanoic acid (0.04%) and Tridecanoic acid (0.03%) in hexane extract of *A.pannosum* and 9, 12-Octadecadienoic (48.50%) > 9-Octadecenoic acid (32.02%) > Hexadecanoic acid (11.48%) > Octadecanoic acid (6.20%) > 9, 12, 15- Octadecatrienoic acid (0.81%) > Eicosanoic acid (0.56%) > 9-Hexadecenoic acid (0.19%) > 11-Eicosenoic acid (0.18%) and Methyl tetradecanoate (0.06%) in hexane extract of *G. tenax*. The phytochemical compounds recognized through GC-MS analysis showed many biological activities are listed in Table 2. While the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The results obtained in the analyses of the hexane extract of *Abutilon pannosum* are listed in below Table 1,

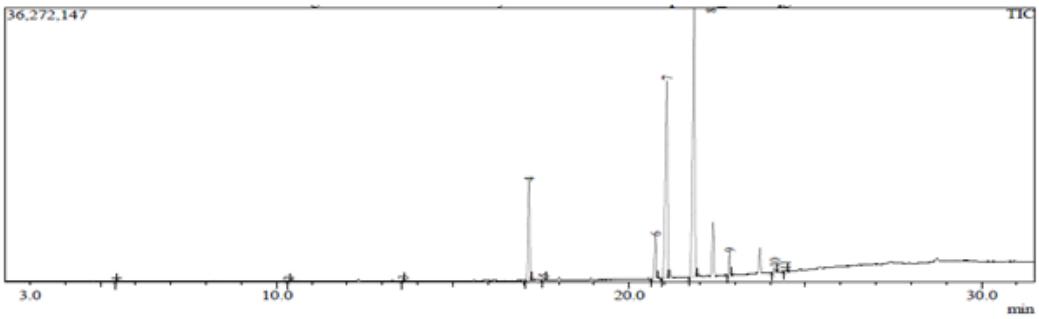

Figure1. *The GC-MS Chromatogram of n-hexane extract of leaves of Abutilon pannosum*

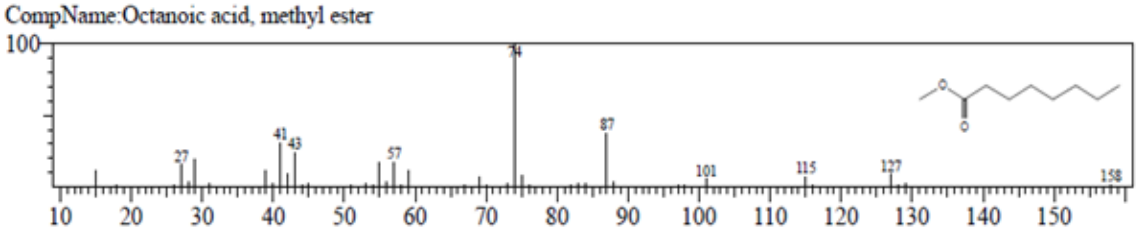

Figure2. *Mass spectrum of Octanoic acid*

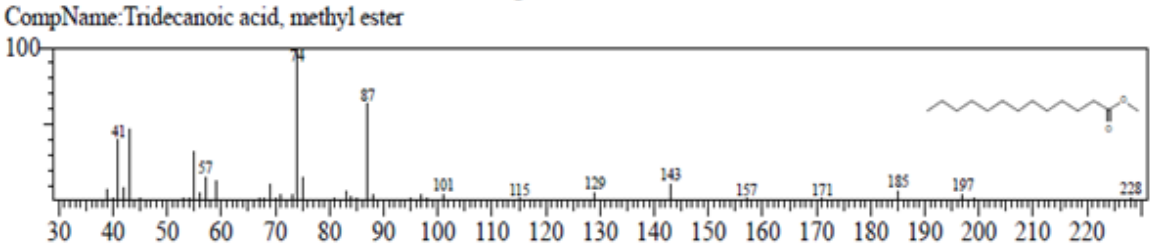

Figure3. *Mass spectra of Tridecanoic acid*

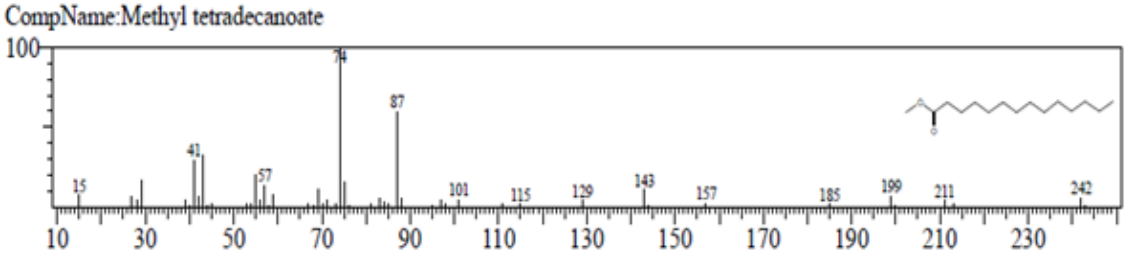

Figure4. *Mass spectrum of Methyl tetradecanote*

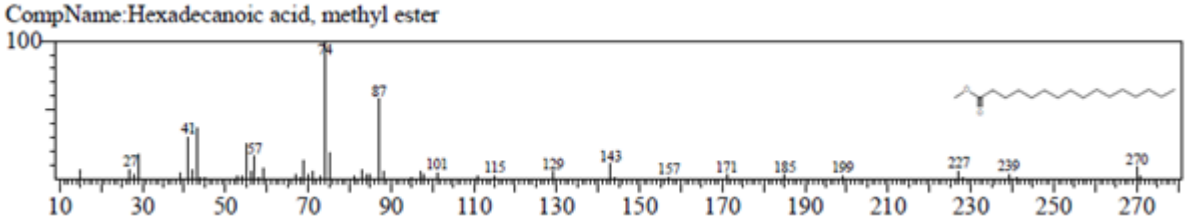

Figure5. *Mass spectrum of Hexadecanoic acid*

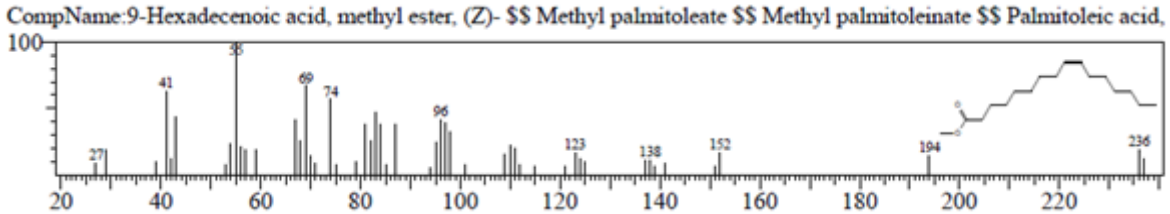

Fig6. *Mass spectrum of 9-Hexadecanoic acid*

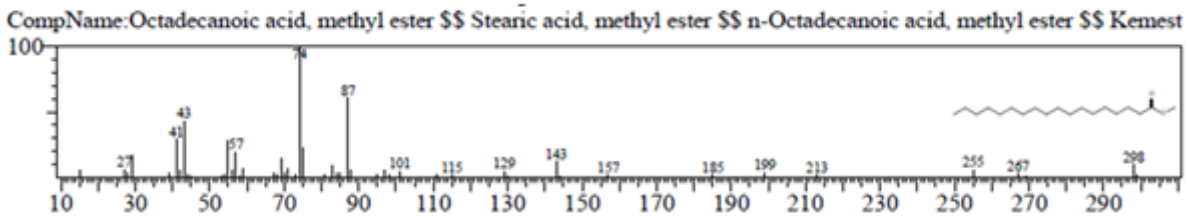

Fig7. *Mass spectrum of Octadecanoic acid*

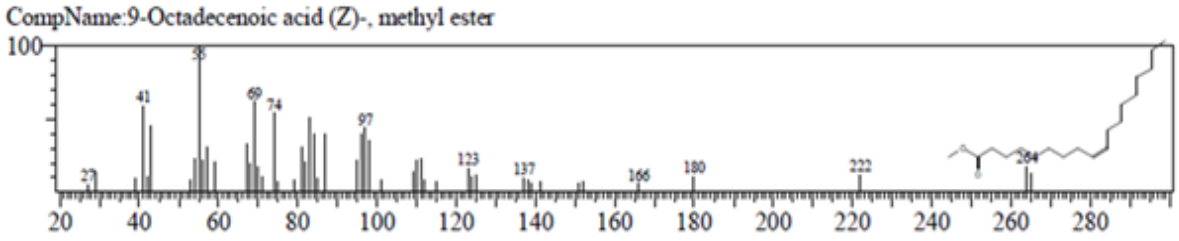

Fig8. *Mass spectrum of 9-Octadecanoic acid (Z)*

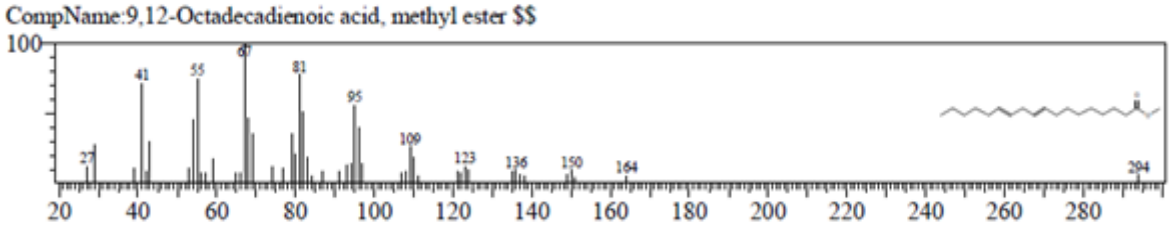

Fig9. *Mass spectrum of 9, 12-Octadecanoic acid*

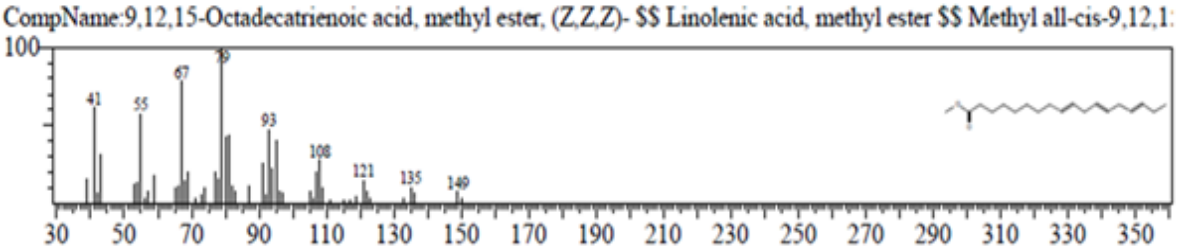

Fig10. *Mass spectrum of 9, 12, 15-Octadecanoic acid*

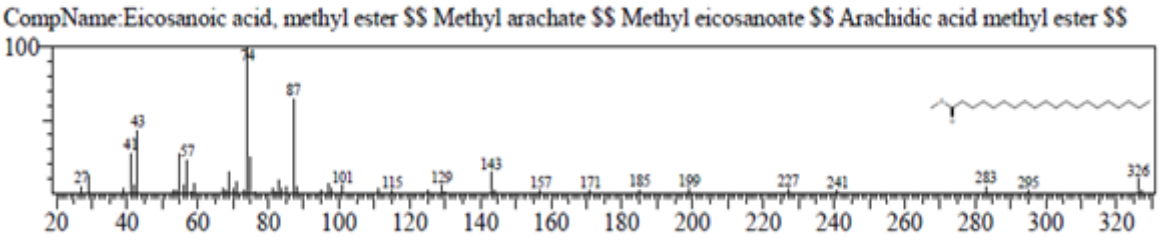

Fig11. *Mass spectrum of Eicosanoic acid*

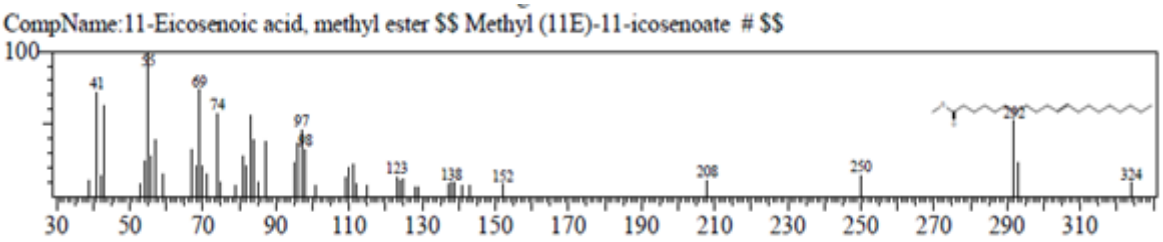

Fig12. *Mass spectrum of 11-Eicosanoic acid*

Table1. *List of Bioactive compound of hexane extract of leaf of Abutilon pannosum*

Sr. No	RT	Area %	H %	A/H ratio	Fatty Acid Methyl Ester	M.F	CAD ID	M. W	SI	Library
1	5.443	0.04	0.11	1.53	Octanoic acid, methyl ester	$C_9H_{18}O_2$	111-11-5	158	94	Nist 27
2	10.331	0.03	0.06	1.90	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	1731-88-0	228	86	Nist27
3	13.584	0.10	0.16	2.43	Methyl tetradecanoate	$C_{15}H_{30}O_2$	124-10-7	242	92	Nist 27
4	17.159	12.12	15.44	3.17	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	112-39-0	270	97	Nist 27
5	17.569	0.22	0.28	3.10	9-Hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	1120-25-8	268	89	Nist 147
6	20.749	5.88	6.71	3.54	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	112-61-8	298	96	Nist 147
7	21.073	33.18	30.8	4.35	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	112-62-9	296	96	Nist 27
8	21.846	45.05	41.98	4.34	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	2462-85-3	294	96	Nist 107
9	22.855	2.62	3.51	3.01	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	301-00-8	292	95	Nist 107
10	24.127	0.60	0.74	3.26	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	1120-28-1	326	96	Nist 107
11	24.431	0.16	0.21	3.01	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	3946-08-5	324	81	Nist 147

According to above results showed that total eleven type fatty acid present in n-hexane extract of *A. pannosum*. It was mainly found to be in order of 9, 12-Octadecadienoic (45.05%) > 9-Octadecenoic acid (33.18%) > Hexadecanoic acid (12.12%) > Octadecanoic acid (5.88%) > 9,12,15-Octadec-atrienoic acid (2.62%) > Eicosanoic acid (0.60%) > 9-Hexadecenoic acid (0.22%) > 11-Eicosenoic acid (0.16%) > Methyl tetradecanoate (0.10%) > Octanoic acid(0.04%) and Tridecanoic acid (0.03%).

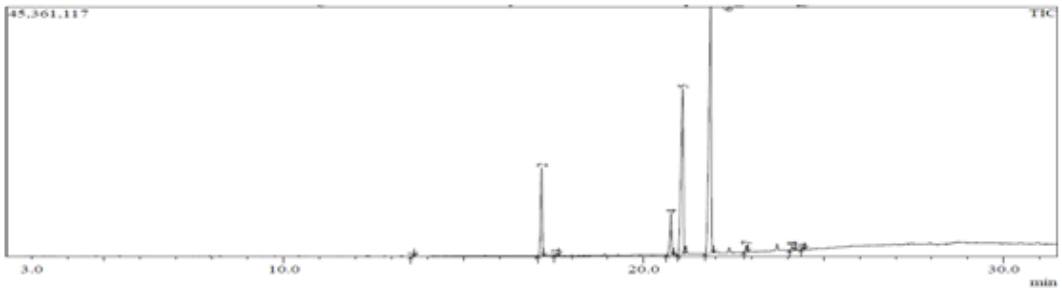

Figure13. *GC - MS Chromatogram of n-hexane extract of leaves of Grewia tenax*

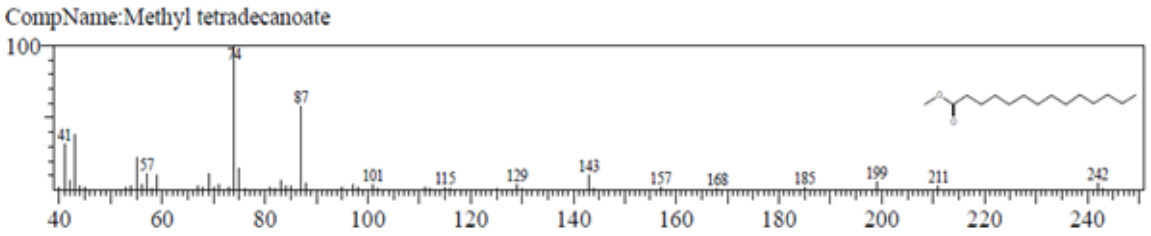

Figure14. *Mass spectrum of Methyl tetradecanote*

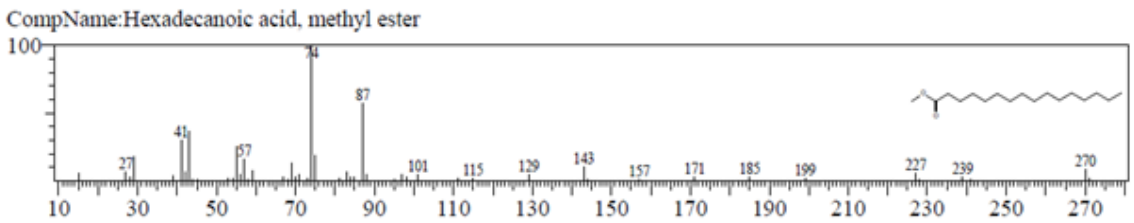

Figure15. *Mass spectrum of Hexadecanoic acid*

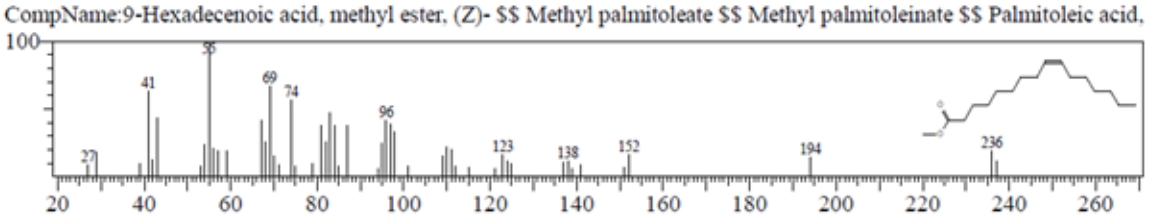

Figure16. *Mass spectrum of 9-Hexadecanoic acid*

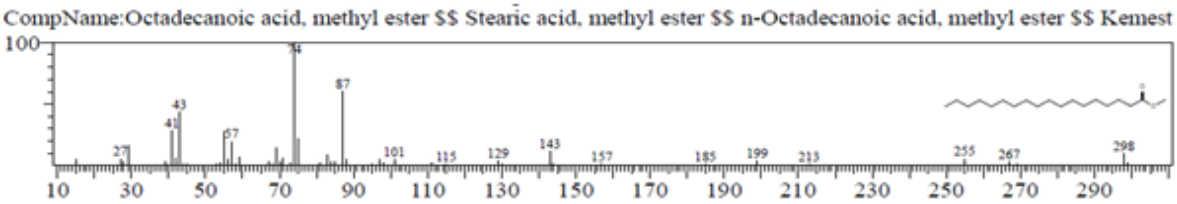

Figure17. *Mass spectrum of Octadecanoic acid*

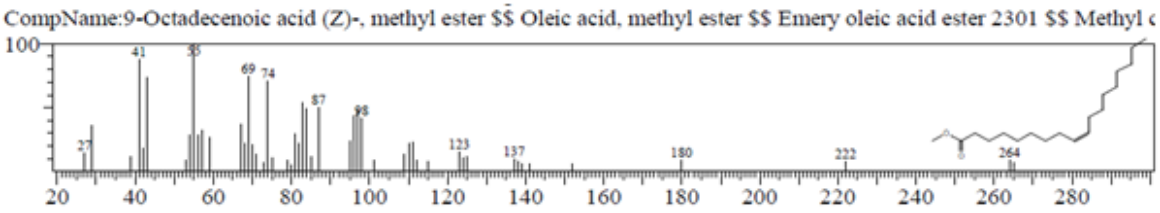

Figure18. *Mass spectrum of 9-Octadecanoic acid*

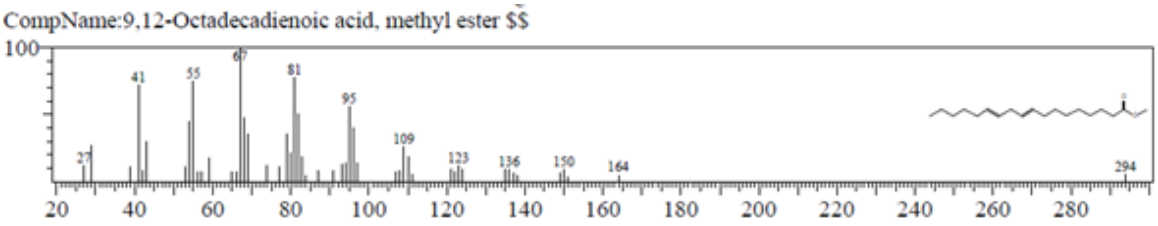

Figure19. *Mass spectrum of 9, 12-Octadecanoic acid*

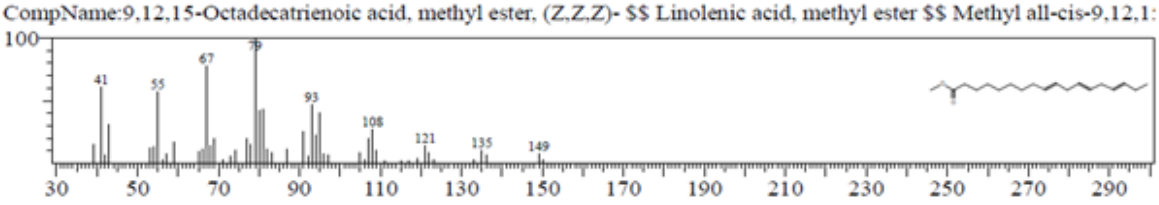

Figure20. *Mass spectrum of 9, 12, 15-Octadecanoic acid*

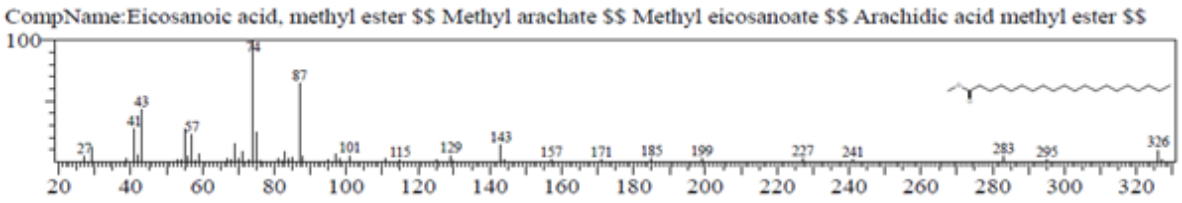

Figure21. *Mass spectrum of Eicosanoic acid*

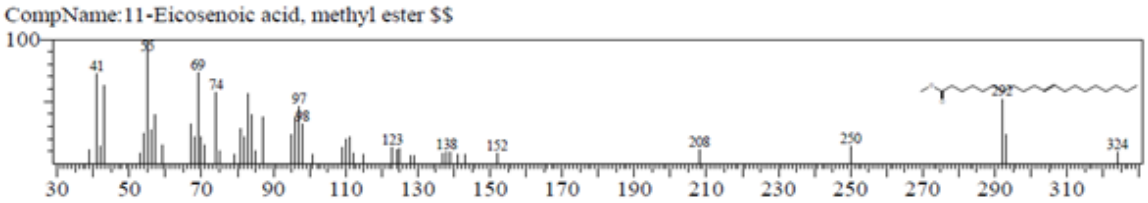

Figure22. *Mass spectrum of 11- Eicosanoic acid*

Table2. *List of Bioactive compound of hexane extract of leaf of Grewia tenax*

Sr. No	RT	Area %	H %	A/H ratio	Fatty Acid Methyl Ester	M.F	CAD ID	M. W	SI	Library
1	13.582	0.06	0.09	2.80	Methyl tetradecanoate	$C_{15}H_{30}O_2$	124-10-7	242	92	Nist27
2	17.166	11.48	15.82	3.16	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	112-39-0	270	97	Nist27
3	17.569	0.19	0.28	2.95	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	1120-25-8	268	89	Nist147
4	20.759	6.20	7.39	3.65	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	112-61-8	298	96	Nist147
5	21.086	32.02	29.8	4.68	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	112-62-9	296	96	Nist27
6	21.863	48.50	44.49	4.75	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	2462-85-3	294	96	Nist107
7	22.855	0.81	1.17	3.00	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	301-00-8	292	95	Nist107
8	24.127	0.56	0.71	3.40	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	1120-28-1	326	96	Nist107
9	24.426	0.18	0.25	3.01	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	3946-08-5	324	81	Nist147

According to above results showed that total eleven type fatty acid present in n-hexane extract of *G. tenax*. It was mainly found to be in order of 9, 12-Octadecadienoic (48.50%) > 9-Octadecenoic acid (32.02%) > Hexadecanoic acid (11.48%) > Octadecanoic acid (6.20%) > 9, 12, 15- Octadecatrienoic acid (0.81%) > Eicosanoic acid (0.56%) > 9-Hexadecenoic acid (0.19%) > 11-Eicosenoic acid (0.18%) and Methyl tetradecanoate (0.06%).

Table3. *Importance of Bioactive compound of hexane extract of leaf of A. pannosum G. tenax*

Sr. No.	Fatty acid name	Nature	Importance	Reference
1	Octanoic acid, methyl ester	Caprylic acid	Candidicide, Flavor, Fungicide, Perfumery, Pesticide	14
2	Tridecanoic acid, methyl ester	Tridecylic acid	Antioxidant, Cancer Preventive, Cosmetic, Hypercholesterolemic, Nematicide, Flavour Ingredient And Lubricant	15
3	Methyl tetradecanoate	Myristic acid ester	Antioxidant, Cancer-preventive, Hypercholesterolemic, Lubricant, Nematicide	16
4	Hexadecanoic acid, methyl ester	Palmitic acid	Antiandrogenic, Nematicide, pesticide, Hypocholesterolemic Hemolytic, Flavor, Antioxidant, Lubricant, Anti-inflammatory, 5-Alphareductase inhibitor, Soap, mosquito larvicide	17, 18, 19,
5	9-Hexadecenoic acid, methyl ester, (Z)-	Palmitoleic acid	Effects of the permeability and partition of ions into 1, 2-dimyristoyl-sn-glycero-3- phosphocholine bilayer at the main phase transition	16
6	Octadecanoic acid, methyl ester	Stearic acid	Antimicrobial activity, Cosmetic , Flavor, Hypo cholesterolemic, Lubricant, Perfumery, Propecic, Suppository	20
7	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid	Anti-Inflammatory, Antiandrogenic, Cancer Preventive , Dermatitigenic, Hypocholesterolemic, 5- Alpha reductase inhibitor, Anemiagenic, Insectifuge, Cosmetic, Flavour, Hypo Cholesterolemic, Lubricant, Perfumery, Propecic, Suppository	14, 15

8	**9,12-Octadecadienoic acid, methyl ester**	**Linoleic acid or Polynoic acid**	**Hepatoprotective, Antihistaminic, Hypocholesterolemic, Antieczemic, Anti-Inflammatory, Antiandrogenic, Cancer Preventive, Dermatitigenic, Irritant, Antileukotriene—D4, Anti-Cancer**	**21, 22**
9	**9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-**	**Polyenoic fatty acid**	**Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antiacne, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic**	**21, 22**
10	**Eicosanoic acid, methyl ester**	**Arachidic acid**	**Alpha-glucosidase inhibitors**	**23**
11	**11-Eicosenoic acid, methyl ester**	**Gondoic acid**	**Antioxidant, Pesticide, Nematicide**	**24**

Fatty acids are important bio compounds which take part in complex metabolic pathways, it having major biological roles. It is use full to construct and repair cell membranes enabling the cells to obtain optimum nutrition and expel harmful waste products and also support the cardiovascular, reproductive, immune and nervous systems, is the production of prostaglandins. These regulate body functions such as heart rate, blood pressure, blood clotting, fertility and play a role in immune system by regulating inflammation. [8-12] The analysis of fatty acid from *A. pannosum* and *G. tenax* by GC/MS showed that it contains various bioactive constituents. In two species presented fatty acid component and its importance were showed above tables. In the present study, except 9, 12-Octadecadienoic and Octadecanoic acid, all the fatty acid contents in *A. pannosum* was found higher than the *G. tenax*. Both plant are very important source of fatty acid. In both plants nine same types of fatty acids were present. They are 9, 12-Octadecadienoic, 9-Octadecenoic acid, Hexadecanoic acid, Octadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Eicosanoic acid, 9-Hexadecenoic acid, 11-Eicosenoic acid, Methyl tetradecanoate, Octanoic acid and Tridecanoic acid. But the concentration has been arrived different. The concentration effects on biological activity of component.

4. CONCLUSION

In the present investigation higher amount bioactive compound have been identified from n-hexane extract of *A. pannosum* (11 types of fatty acid) and *G. tenax* (9 types of fatty acid) by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds in both species proved the medicinal importance. In both plants nine types of bioactive compounds were found same. In the present study, except 9, 12-Octadecadienoic and Octadecanoic acid, all the fatty acid contents in *A. pannosum* was found higher than the *G. tenax*. Though, further studies might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be create a new way to treat many incurable diseases.

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