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GS-MS Study, Antimicrobial and Antioxidant Activity of Fixed Oil from *Ximenia Americana* L. Seeds

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ABSTRACT

The aim of the present study is to investigate the chemical constituents of the Essential Oil from Ximenia americana L. plant Seeds and to evaluate its antioxidant potential and antibacterial activity. Using Soxhlet method to extract the essential oil from Ximenia americana L. plant Seeds. The chemical constituents of Ximenia americana Oil were identified and quantified by GC-MS, where paper disc diffusion assay was employed to evaluate the antibacterial activity and Antioxidant activities were evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity Twentythree components has been identified. Ten of them are major namely 9-Octadecenoic acid (Z)-, methyl ester (32.40%), Methyl octadeca-9-yn-11-trans- enoate (13.08%) , 9,12-Octadecadiynoic acid, methyl ester (2.69%) , 15-Tetracosenoic acid, methyl ester, (Z)- (4.99 %) , , 10-Nonadecenoic acid, methyl ester (6.81%), Hexacosanoic acid, methyl ester (4.12%), Tetracosanoic acid, methyl ester (3.02%), Cyclopropaneoctanoic acid, 2-octyl-, methyl ester (15.93%), Octacosanoic acid, methyl ester2.38%) and cis-10-Nonadecenoic acid, methyl ester (7.32%). the antibacterial showed showed low inhibitory effect against Pseudomonas aeruginosa (9mm), Bacillus subtilis (10mm), albicans (10mm) and Escherichia coli (11mm) and Candida albicans. The DPPH assay, showed moderate antioxidant potential (49, 0.01 compared with standard 91± 0.01;

Keywords: *GC-MS Analysis, Antibacterial and Antioxidant Activities*

INTRODUCTION

Flora of Sudan is rich with 3140 species of flowering plants belonging to 170 family and 1280 genera [1], including the progenitors of many important food crop of today. Some medicinal species were well known as the basis of European herbal medicine.

Many of these plants were threatened where other disappeared. *Ximenia americana L*. plant (X.a) belong to Family Olacaceae, (known as Umedica), is spiny shrub or tree up to 6m [2]. The fruits of the plant up to 3cm long, light green turning to yellow, orange or red onripening, containing a small embryo; and they have up to 60% oil [3].

Phytochemical screening of the extract of the plant revealed the present of saponins, cyanogenic glycosides, flavonoids and tannins [4], also the extracts of (X.a) showed an antioxidant activity [5]. In Sudan, the different parts of the plant extraction are used in folk medicines as: leaves and twigs used to treatment of fever, colds, mouthwash for toothaches, laxative and eye lotion [6].

The extract of the leaves used for headaches and poison antidote, where roots used for skin aches, headaches, leprosy, hemorrhoids, sexually transmitted diseases, guinea worm, sleeping sickness and as poison antidote [7].

The bark and fruits applied skin ulcers, treating habitual constipation, used as vermifuge and it's eaten [8]. Ximenia americana L. used for treatment of irregular menstruation, rheumatism and cancer [9], and for treatment diarrhea [10]. Investigation showed that the constituents of (X.a) have showed several biological activities such as antimicrobial, antifungal, anticancer and

antioxidant, and the antimicrobial activity of the extract of (X.a) appeared to be due to the presence of secondary metabolites such phenol, terpenes, tannins and glycosides [11,4)

MATERIALS

Plant Material

Ximenia americana fruits were collected from forest around Almoglad city-West Kordofan state – Sudan - on January 2019. The plant was authentically by Dr. Ahmed Suliman - Gum and Forest Products Research Center - West Kordofan University - Alnohud - Sudan.

Extraction of Oil

100 g of the seeds were ground into fine powder. Powdered seeds were extracted with n-hexane using Soxhlet extractor for six hours. The volume of hexane was reduced under reduced pressure. The oil of *Ximenia americana* (L) was obtained by evaporating the reduced hexane by air drying in a steady current. The oil was kept in a refrigerator for further manipulation.

GC /MS Method

The qualitative and quantitative analysis of the sample was carried out by using GC MS technique model (GC /MS-QP2010-Ultra) from japan "Simadzu Company, with capillary column (Rtx-5ms -30 m × 0.25 mm ×0.25 µm).

The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60 C with rate 10 C /min to 300 c as final temperature degree, the injection port temperature was 300c, the ion source temperature was 200 c and the interface temperature was 250 c.

The sample was analyzed by using scan mode in the range of m/z 40 – 550 charge to ratio. Identification of component for the sample was achieved by comparing their retention times and mass fragmentation patent with those available in the library, the National Institute of Standards and Technology (NIST), results were recorded.

Antimicrobial Assay

Disc diffusion method: The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [13]. Bacterial suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5).

One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

DPPH Radical Scavenging Assay

The DPPH radical scavenging was determined according to the method of [14] with some modification. In 96 –well plate, were allowed to react with 2, 2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 C. The concentration of DPPH was kept as (300µL M).

The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using mutilate reader spectrophotometer. Percentage radical scavenging activity by sample was determine in comparison with DMSO treated control group all testes and analysis were run in triplicate.

RESULTS AND DISCUSSIONS

Constituents of Ximenia Americana L Seed Oil

The typical GC chromatogram of oil extracted from Ximena Americana L.Seeds revealed the presence of twenty three components was presented in **Fig. 1** and **Table. 1**.

Ten of them are major namely 9-Octadecenoic acid (Z)-, methyl ester (32.40%), Methyl octadeca-9-yn-11-trans- enoate (13.08%) , 9,12-Octadecadiynoic acid, methyl ester (2.69%) , 15-Tetracosenoic acid, methyl ester, (Z)- (4.99 %), 10-Nonadecenoic acid, methyl ester (6.81%), Hexacosanoic acid, methyl ester (4.12%), Tetracosanoic acid, methyl ester (3.02%), Cyclopropaneoctanoic acid, 2-octyl-, methyl ester (15.93%), Octacosanoic acid, methyl ester2.38%) and cis-10-Nonadecenoic acid, methyl ester (7.32%).

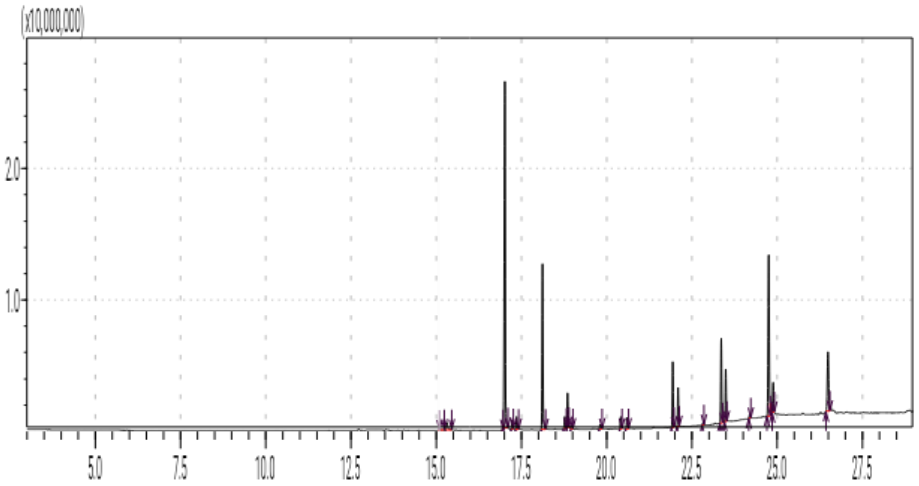

Figure1. *The typical GC chromatogram of Ximenia americana L seeds oil.*

The peak appeared at 17.015 min with area (32.40%) and has MS ions m/z **337 [**M] $^{+}$ correspond to formula $C_{19}H_{36}O_2$**Figure 2** as well and **55** (base peak) which are in agreement with 9-Octadecenoic acid (Z)-, methyl ester. The peak of Methyl octadeca-9-yn-11-trans- enoate is appeared at 18.120 min with area (13.08%) and has MS ions m/z **346 [**M] $^{+}$ correspond to formula $C_{19}H_{38}O_2$**Figure 3**. **Figure (4)** shows the mass spectrum for 9,12-Octadecadiynoic acid, methyl ester which appeared at 18.851 min in total ion chromatogram with area (2.69%) this compound has MS ions m/z **458** [M] + correspond to formula $C_{19}H_{30}O_2$. The peak at R.T. 21.937 min with Area (4.99 %) on GC chromatogram, produced molecular ion peaks *m/z* at 4**26** [M] $^{+}$ corresponded to formula $C_{25}H_{48}O_2$ in it MS spectra **Figure 5,** which are in agreement with 15-Tetracosenoic acid, There are another peaks appeared at 22.093 , 23.359 , 23.493 , 24.753 , 24.885, and 26.490 mint with Area (6.81, 4.18,3.02,15.93,2.38 and 7.32 %) respectively Figures **6**, **7, 8, 9 , 10** and **11** produced molecular ion peaks m/z at 381 ,510,488, 530,455 and 536 $[M]^{+}$ corresponded to formula $C_{19}H_{36}O_2$, $C_{27}H_{54}O_2$, $C_{25}H_{50}O_2$, $C_{20}H_{38}O_2$ $C_{29}H_{58}O_2$ and $C_{20}H_{38}O_2$ in their MS spectra Figure **5**, **6** and **7**, in addition these compounds identified to be 10-Nonadecenoic acid, methyl ester , Hexacosanoic acid, methyl ester, Tetracosanoic acid, methyl ester, Cyclopropaneoctanoic acid, 2-octyl-, methyl ester , Octacosanoic acid, methyl ester and cis-10-Nonadecenoic acid, methyl ester .

Table1. *Chemical constituents of Ximenia americana L Seeds Oil*

Peak report TIC					
ID	**RT**	**M+**	**BP**	**Key fragment ions**	**Name of compound**
1	15.084	268	41	55 69 74 87 96 123 152	7-Hexadecenoic acid, methyl ester, (Z)-
2	15.126	268	55	69 87 98 194 236	9-Hexadecenoic acid, methyl ester, (Z)-
3	15.313	270	74	41 87 129 143 227	Hexadecanoic acid, methyl ester
4	16.956	294	67	41 55 81 95 109 123263	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
5	17.015	296	55	67 97 123 180 222 264	9-Octadecenoic acid (Z)-, methyl ester
6	17.220	298	74	43 57 87 143 199 255	Methyl stearate
7	17.355	296	55	41 69 87 123 180 222 264	10-Octadecynoic acid, methyl ester
8	18.120	292	79	41 55 93 150 164 217 261	Methyl octadeca-9-yn-11-trans-enoate
9	18.772	324	55	69 83 97 208 250 263 292	cis-11-Eicosenoic acid, methyl ester
10	18.851	294	67	41 55 81 95 150 223 263	9,12-Octadecadiynoic acid, methyl ester
11	18.971	326	74	43 57 87 143 185 199 283 297	Eicosanoic acid, methyl ester
12	19.805	292	79	41 55 67 93 108 135 163 236	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
13	20.414	352	55	41 69 81 123 236 278 320	13-Docosenoic acid, methyl ester, (Z)-
14	20.591	354	74	43 57 87 143 185 269 323	Docosanoic acid, methyl ester
15	21.937	380	55	41 69 83 125 152 250 306 348	15-Tetracosenoic acid, methyl ester, (Z)-
16	22.093	382	74	41 43 57 129 143 383 339	Tetracosanoic acid, methyl ester
17	22.825	354	74	323 143 87 57 43 41	Methyl (Z)-5,11,14,17-eicosatetraenoate
18	23.359	310	55	41 69 83 97 123 150 263 278	10-Nonadecenoic acid, methyl ester

19	23.493	410	74	43 55 87 143 199 255 311 367	Hexacosanoic acid, methyl ester
20	24.192	292	79	55 67 93 121 150 164 261	Methyl octadeca-9-yn-11-trans-enoate
21	24.753	310	55	41 69 139 153 180 194 236 278	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester
22	24.885	438	74	43 57 143 199 297 353 396	Octacosanoic acid, methyl ester
23	26.490	310	55	41 69 97 165 180 194 236 278	cis-10-Nonadecenoic acid, methyl ester

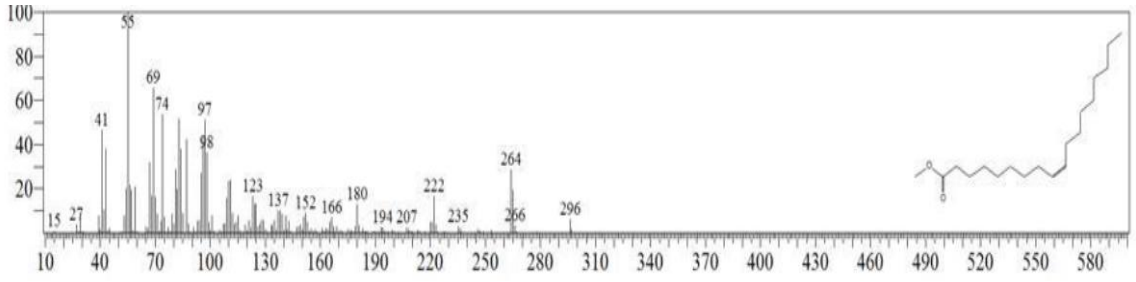

Figure2. *The Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester*

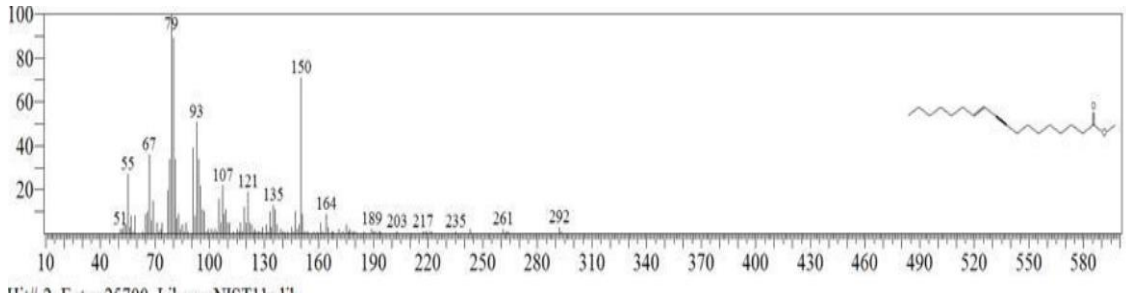

Figure3. *The Mass spectrum of Methyl octadeca-9-yn-11-trans-enoate*

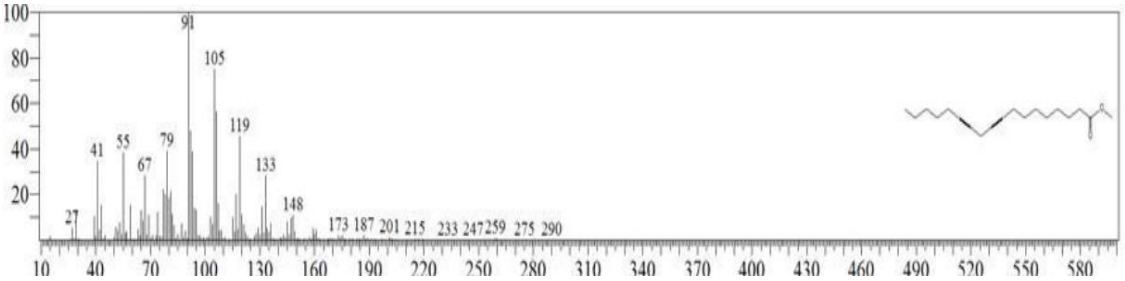

Figure4. *The Mass spectrum of 9,12-Octadecadiynoic acid, methyl ester*

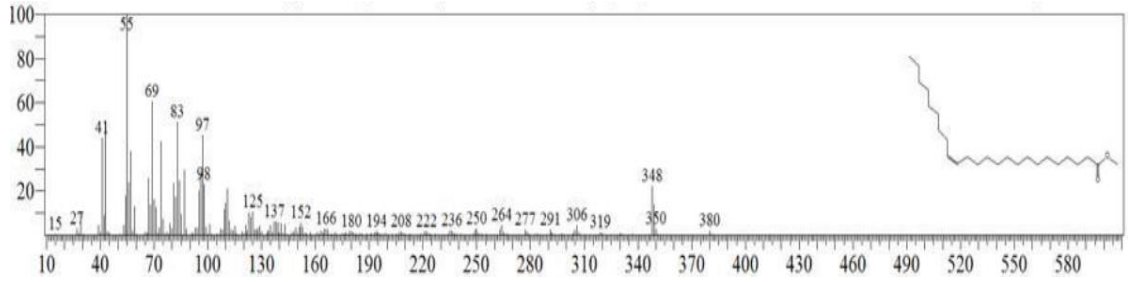

Figure5. *The Mass spectrum of 15-Tetracosenoic acid, methyl ester, (Z)-*

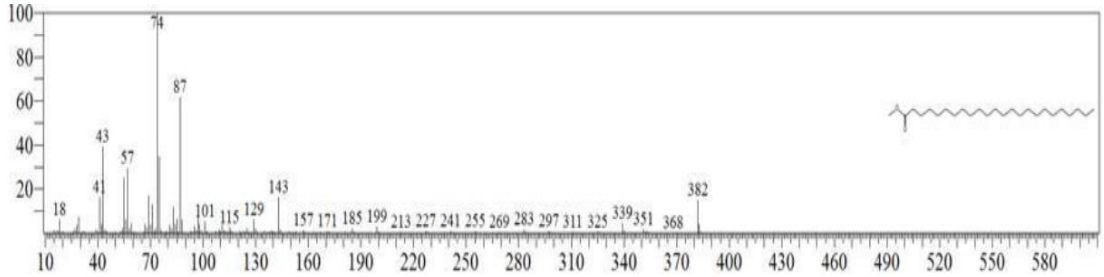

Figure6. *The Mass spectrum of Tetracosanoic acid, methyl ester*

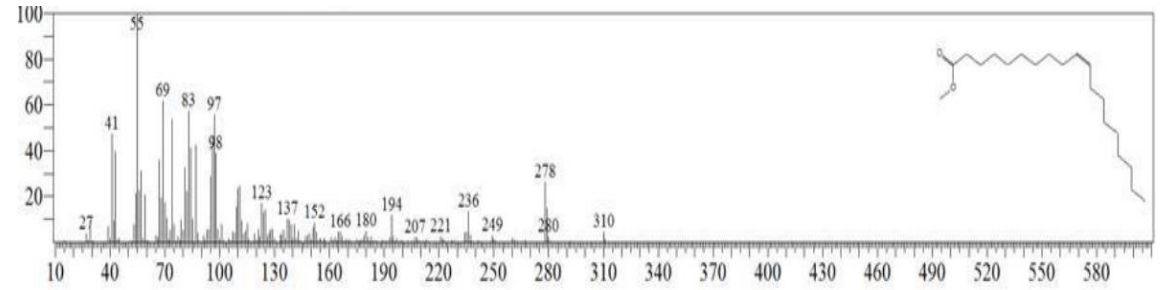

Figure7. *The Mass spectrum of 10-Nonadecenoic acid, methyl ester*

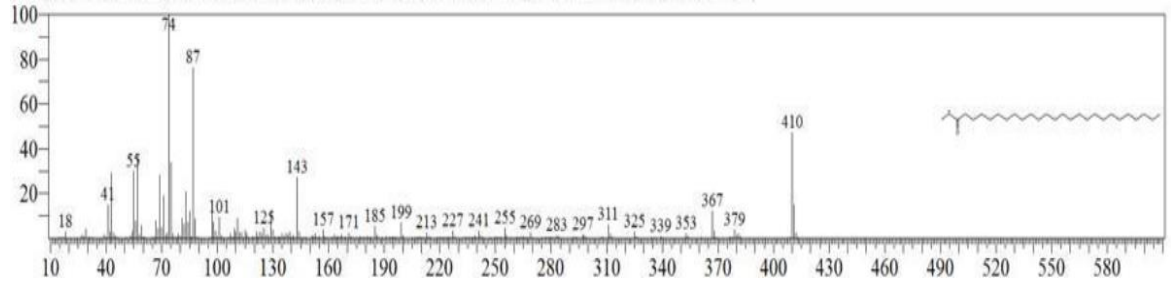

Figure8. *The Mass spectrum of Hexacosanoic acid, methyl ester*

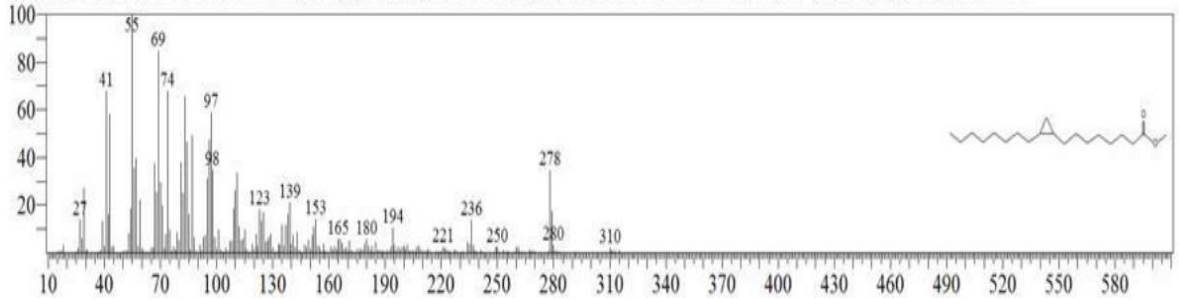

Figure9. *The Mass spectrum of Cyclopropaneoctanoic acid, 2-octyl-, methyl ester*

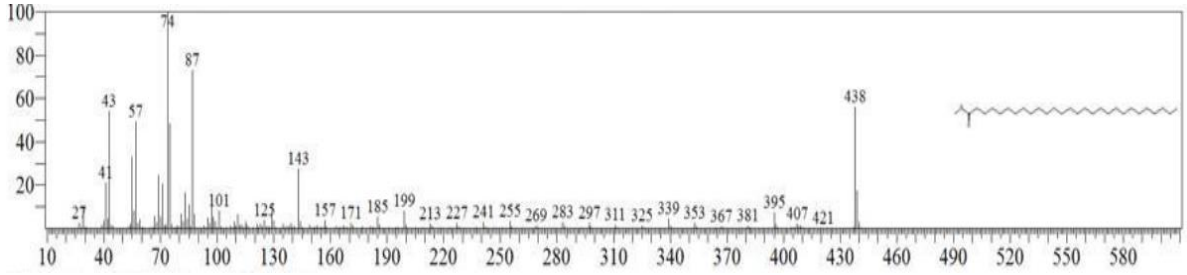

Figure10. *The Mass spectrum of Octacosanoic acid, methyl ester*

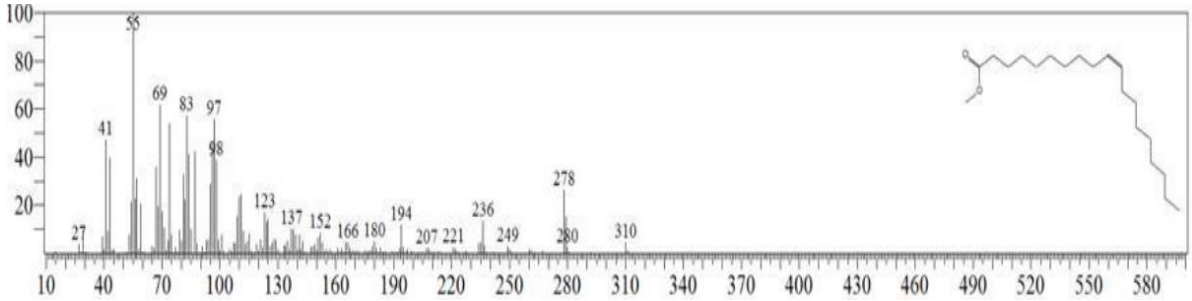

Figure11. *The Mass spectrum of cis-10-Nonadecenoic acid, methyl ester*

The present work reveals that the extract ***of*** *Ximenia americana L* showed moderately activity against microorganisme Are given in **Table (2)** and The DPPH assay showed antioxidant potential (49± 0.01) compared with standard (91± 0.01). Are given in **Table (3)** is a good source of antioxidants due to the presence of fatty acids compounds, also is a potential source of natural antibacterial, and justify its uses in folkloric medicines. In conclusion, the

results showed that the Oïl of *Ximenia americana L* a potential source of natural antibacterial, antioxidant potential and justify its uses in folkloric medicines.

Table2. *Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)*

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ca	Ps.
Sample	100	10	10	10	-	9

(Ba = Bacillus subtilis, Ec = Escherichia coli, Pa = Pseudomonas aeruginosa, Ca = Candida albicans, Bs = Bacillus subtilis)

Table3. *Antioxidant activity of Ximenia americana L seeds oil.*

ID	Sample	RSA ± % SD (DPPH)
Sample	Oil	49 ,01
Standard	Propyl Gallate	91,.01

Conclusion

Our results showed that the Fixed oil extracted from *Ximenia americana L seeds* rich with various fatty acids derivatives, phenolic compounds, Steroidal derivatives and pentacyclic triterpenes. The existence of these bioactive chemical compounds proved the use of this plant for various ailments by traditional medical practitioners. The Ximenia americana L seeds fixed oil have significant antibacterial and antioxidant capacity, suggesting to use in flavoring agent, food industry and medicinal purposes.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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