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GC-MS Analysis of *Rhus Tripartita* Roots Extract

Zaid M. Najah[1]
zmnajah@elmergib.edu.ly

Madeha R. Alshawish [2]
Mralshawish@elmergib.edu.ly

[1] *Associate Professor, Chemistry Department, Science Faculty, ElMergibUniversity, Alkoms, Libya.*
[2] *Assistant Professor, Chemistry Department, Arts and Science Faculty, ElMergibUniversity, Gaser Khair, Libya.*

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الملخص

نبات الجداري نبات معروف في ليبيا و شمال افريقيا و يستخدم بكثرة في الطب الشعبي الليبي، وهو نبات عشبي ينبت في البيئة الصخرية وينتمي لعائلة الكاجو .تم تحليل المستخلص الايثانولي لمسحوق جذور النبات بإستخدام جهاز كروماتوجرافي الغاز المتصل بمطياف الكتلة حيث تم التعرف على ثلاثة و ستين مركبًا ، نصفها إسترات لأحماض دهنية ، ستة كحولات طويلة السلسلة، ثلاثة كيتونات، ثلاث ألكينات طويلة السلسلة، إثنان من الفايتوستيرولات، أربع مركبات حلقية غير متجانسة الحلقة، أربع أحماض كربوكسلية، إثنان من كلوريدات الاحماض ومركبين من السلفونات. أكثر المركبات نسبة في المستخلص كانت (9Z,12Z) Octadecadienoyl chloride (9E,12E) Octadecadienoyl chloride, (E)-9-Octadecenoic acid methyl ester و بنسبة 46.89 %، تليها مركبات Hexadecanoic acid methyl ester, 14-Methyl Pentadecanoic acid methyl ester و بنسبة 11.62%، البحث أوضح أن مستخلص جذور النبات غني جدًا بالمركبات العضوية النشطة حيوياً و هذا مايفسرالاستعمال الواسع لهذا النبات في الطب الشعبي.

Abstract

Rhus Tripartita is a rocky bush belongs to *Anacardiaceae* family, well known for uses in folk medicine in north Africa, and known locally in Libya with Jdari name. The ethanolic extract of dried roots powder was analyzed using GC-MS. Sixty-three compounds were identified, which half of them are fatty acid esters, six long chain alcohols, three ketones, three long chain alkenes, two phytosterols, four heterocyclic compounds, four carboxylic acids, two acid chlorides and two sulfones. The major components of the extract were (9*Z*,12*Z*) Octadecadienoyl chloride, (9*E*,12*E*) Octadecadienoyl chloride, (*E*)-9-Octadecenoic acid methyl ester with peak percent of 46.89%, and Hexadecanoic acid methyl ester, 14-Methyl Pentadecanoic acid methyl ester with peak percent of 11.62 %. The extract of *Rhus Tripartita* is very rich with bioactive organic components which may explain the extensive usage in traditional medicine.

Keywords: *Rhus Tripartita*, GC analysis, Fatty acid esters, Bioactive compounds.

Introduction

The fast rising of drug discovery research and drugs industry still not enough to find all cure for chronic deceases, mother nature still donates unique cures and offer sustainable source of magic molecules, and that's explains the importance of folk medicine in the present time (1). Due to the divergent geographical location in the middle of North Africa, Libya owns wide range of medicinal plants, especially in the northern coast, *Rhus Tripartita* (known locally as Jdari) is one of the well-known medicinal plants in Libya and Mediterranean region and still in use in

traditional medicine especially roots part, researches revealed good anti diarrhea, anti-ulcer properties of the roots extract (2), additionally, roots extract was found to exhibit anti tumorigenic, hypoglycemic, antimicrobial activity (3).

Rhus tripartita is a rocky push from *Anacardiaceae* family. The shrub is very branched of 1.5 to 4 m height characterized with a large stump as shown in Figure 1. The wood is very dense, dark red in color, the inflorescence is in cyme, bearing yellow flowers, the roots are highly developed, both in the depth and laterally, very strong and dark red in color (4).

Figure 1. *Rhus Tripartita plant*

Discovering the exact chemical constituents of plant roots became a necessity, GC-MS is a very useful and informative tool, affording chemical structure of all possible organic compounds present in the extract. In this research, investigation of ethanolic extract of *R. Tripartita* roots was carried out in order to identify the exact chemical structure of bioactive components responsible for biological activity of the plant roots.

Materials and Methods

Plant Material

Roots of *Rhus Tripartita* plant were collected from south of Gaser Khiar rocky region (32°46'26"N 13°46'56"E, 75 km east of the capital Tripoli) in summer of 2020. The roots were identified by plant taxonomist from Botany department, Arts and Science Faculty, Elmergib University. The roots material was cleaned and foreign materials were removed, grinded to a fine powder using electrical blender, the powder was stored in airtight container and stored at room temperature until further use.

Sample Preparation for GC Analysis

10.0 ml of methanol was added to a homogenized powder sample (2.0 g), the mixture was shaken vigorously for 60 min. to transfer phytochemicals from the sample matrix into the organic layer. The extract was Centrifuged and the supernatant was collected and filtered through 0.2 µm syringe to remove particulate matter. The filtered extract was concentrated using

rotary evaporation. The dried, concentrated extract was dissolved in 5.0 ml ethanol, then, 1 µL of reconstituted sample was injected into the GC injection port using a microliter syringe.

GC-MS Analysis Method

The analysis of chemical composition of roots powder ethanolic extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 °C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290 °C by 30 °C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260 °C respectively; Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min. and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Results and Discussion

Gas chromatography (GC) is an analytical technique applicable to gas, liquid, and solid samples especially compounds that are evaporated by heat. If a mixture of compounds was analysed using GC, each compound can be separated and quantified. Advantages of gas chromatography are more than be counted, high sensitivity, GC can detect even trace amounts of compounds in a mixture, making it a powerful tool in analytical chemistry. The high sensitivity of GC is due to the use of small sample sizes and the efficient separation of compounds. Time saving also is a big advantage, GC is very fast and informative analysis tool, in addition to sample size use and easy preparation sample method before analysis.

Analysis of ethanolic extract using GC-MS column showed wide range of organic compounds, retention times, compound names, peak area percentage, molecular formulas and molecular weights are shown in Table 1, The chromatogram of is depicted in Figure 2.

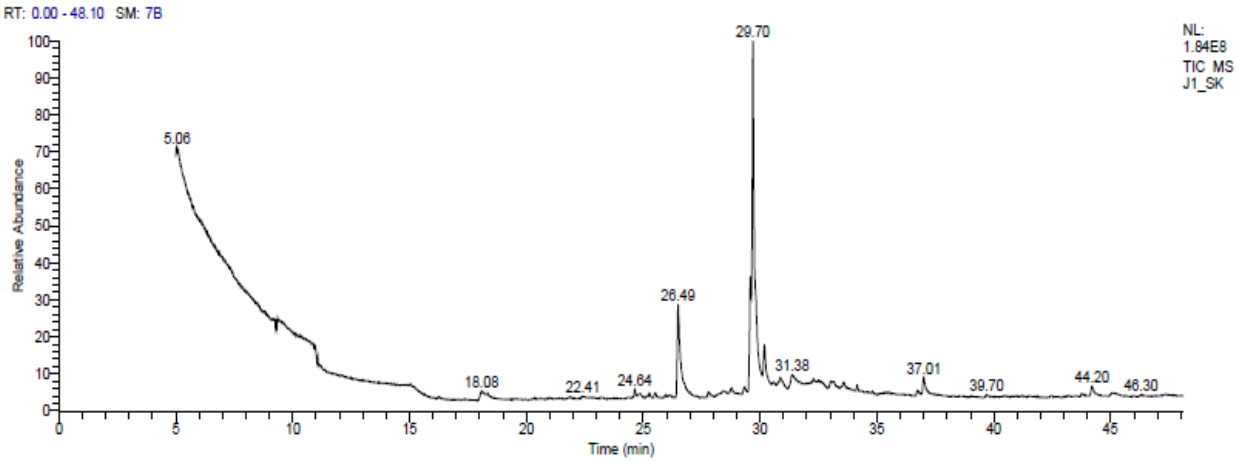

Figure 2. GC Chromatogram of *R.Tripartita* roots ethanolic extract

These phytochemical compounds prediction is based on the phytochemical and ethnobotanical databases of NIST library. To the best of our knowledge, there is no reported research dealt with GC analysis of roots material of studied plant.The compounds shown in the Table 1 are very diverse; fatty

acids and their esters, hydrocarbons, alcohols, ketones, heterocycles, carboxylic acid halides and phytosterols.

Table 1. Identified compounds in the ethanolic extract of *R. Tripartita* plant roots

No.	RT	Compound Name	Area %	MW	MF
1	5.06	Trichloromethane	2.04	118	$CHCl_3$
2	5.06	Dichloro methyl ethyl sulfone	2.04	176	C3H6Cl2O2S
3	5.06	Oxybis[dichloro}- Methane	2.04	182	C2H2Cl4O
4	9.24	3,4-Dihydrothieno-(3,4-B)-5-Carboxy Thiophene	0.79	186	C7H6O2S2
5	9.39	2-((2-Hexoloxy)Ethoxy) Ethanol	1.00	190	C10H22O3
6	9.44	3-Oxo-20-Methyl-11-β-Hydroxyconanine -1,4-Diene	1.35	341	C22H31NO2
7	10.88	4,5,5-D3-Trans-3,4-Dihydroxy Cyclopentene	3.02	103	C5H5D3O2
8	18.07	15-Methyl tricyclo[6.5.2(13,14).0(7,15)]Pentadeca-1,3,5,7,9,11,13-Heptene	1.24	206	C16H14
9	18.07	5- Methyl tricyclo [6,2,1.0(2,7)]Undeca-4,9-Diene-3,6,-Diol	1.24	206	C13H18O2
10	18.07	Octahydro-1-methyl-1-(2-Propenyl))-, (1β,4Aβ,8Aβ)- 2(1H)-Naphthalenone	1.24	206	C14H22O
11	24.64	13-Heptadecyn-1-ol	0.76	252	C17H32O
12	24.64	(Z)-9-Octadecenoic acid	0.76	282	C18H34O2
13	24.64	2- (Z)-9- Octadecenyloxy Ethanol	0.76	312	C20H40O2
14	25.27	Tridueteriomethyl-10-Epoxy-7-Ethyl-3,11-Dimethyl-Trideca-2,6-Dienoate	0.58	297	C18H27D3O3
15	25.27	6,9,12-Octadecatrienoic acid, Methyl ester	0.58	292	C19H32O2
16	25.27	2-(7-Heptadecynoloxy) Tetrahydro-2H-Pyran	0.58	336	C22H40O2
17	25.27	2-Butyloxycarbonyloxy-1,1,10-Trimethyl-6,9-epidioxydecalin	0.58	326	C18H30O5
18	25.52	(all Z)-5,8,11,14-Eicosatetraenoic acid, Methyl ester	0.59	318	C21H34O2
19	25.52	6,9,12,15-Docosatetraenoic acid, Methyl ester	0.59	346	C23H38O2
20	26.49	Hexadecanoic acid, Methyl ester	11.62	270	C17H34O2
21	26.49	14-Methyl- Pentadecanioc acid, Methyl ester	11.62	270	C17H34O2
22	27.78	Hexadecanoic acid, ethyl ester	0.64	284	C18H36O2
23	27.78	2-Acetyl-3-(2-Cinnamedo)ethyl-7-MethoxyIndole	0.64	362	C22H22N2O3

24	27.78	Oleic Acid	0.64	282	C18H34O2
25	28.77	Hexadecanioc Acid, 2,3-Dihydroxy propyl Ester	0.64	330	C19H38O4
26	28.77	Hexadecanioc acid, 1-(Hydroxymethyl)-1,2-Ethandiyl Ester	0.68	568	C35H68O5
27	28.77	Derivative of Tris Trimethyl Silyl ether	0.68	644	C37H68O3Si3
28	28.77	Hexadecanioc Acid, 2-Hydroxy-1,3-Propanediyl Ester	0.68	568	C35H68O5
29	29.33	Trideuteriu methyl 10-Epoxy-7-Ethyl-3,11-dimethyl trideca-2,6-Dienoat	0.82	297	C18H27D3O3
30	29.33	12-Methyl-E,E-2,13-octadecadien-1-ol	0.82	280	C19H36O
31	29.33	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester	0.82	436	C25H40O6
32	29.58	(Z,Z)-9,12-Octadedienoic acid methyl ester	9.92	294	C19H34O2
33	29.70	(Z,Z)-9,12-Octadecadienoyl Chloride	46.89	298	C18H31ClO
34	29.70	(9*E*,12*E*)-9,12-Octadecadienoyl Chloride	46.89	298	C18H31ClO
35	29.70	(*E*)-9-Octadecenoic acid, methyl ester	46.89	296	C19H36O2
36	30.18	Octadecanioc acid, methyl ester	3.84	298	C19H38O2
37	30.18	Cyclopentane tridecanoic acid, methyl ester	3.84	296	C19H36O2
38	30.18	*Trans*-Cyclopropanepentanoic acid, 2-undecyl, methyl ester	3.84	310	C20H38O2
39	30.18	Cyclopropane dodecanoic acid, 2-octyl-, methyl ester	3.84	366	C24H46O2
40	30.18	Heptadecanoic acid, 16-methyl-, methyl ester	3.84	298	C19H38O2
41	30.87	Hi-Oleic Safflower oil	1.65	450	C21H22O11
42	31.37	9,12-Octadecadienoic acid (*Z*,*Z*)-, 2-hydroxy-1-(hydroxymethyl) ethyl Ester	1.70	354	C21H38O4
43	33.03	14-Isopropyl-1,13-dimethoxy-Podocarpa-1,8,11,13-tetraen-3-One	0.84	342	C22H30O3
44	33.03	1,3-Dioxolane-4-Methanol, 2-Pentadecy Acetate	0.84	356	C21H40O4
45	33.03	(6β,16β)-6,16-dimethyl- Pregn-4-ene-3,20-dione	0.84	342	C23H34O2
46	33.15	13-Heptadecyn-1-ol	0.82	252	C17H32O
47	33.15	(3β,5β)- 2-methylene-Cholestan-3-ol	0.82	400	C28H48O
48	33.58	*Trans*-Oxiraneundecanoic acid, 3-pentyl-,	0.91	312	C19H36O3

		methyl ester			
49	**33.58**	***Cis*-Oxiraneundecanoic acid, 3-pentyl-, methyl ester**	**0.91**	**312**	**C19H36O3**
50	**34.16**	**2-Hydroxy-3-[(9E)-9-Octadecenoyloxy]Propyl (9E)-9-Octadecanoate**	**0.59**	**620**	**C39H72O5**
51	**34.16**	**3,4',5,6'-tetrakis(1,1-dimethylethyl)-[1,1'-Biphenyl]-2,3'-diol**	**0.59**	**410**	**C28H42O2**
52	**34.16**	**(*E*,*E*,*E*) 9-Octadecenoic acid -1,2,3-propanetriyl ester**	**0.59**	**884**	**C57H104O6**
53	**34.16**	**3-(1,5-Dimethylhexyl)-3a,10,10,12 b-tetramethyl-1,2,3,3a,4,6,8,9,10,10 a,11,12,12a,12b-tetradecahydro-ben zo[4,5]cyclohepta[1,2-E]indene**	**0.59**	**410**	**C30H50**
54	**36.74**	**Cyclopropanedodecanoic acid, 2-octyl-, methyl ester**	**0.71**	**366**	**C24H46O2**
55	**36.74**	**Docosanoic acid, methyl Ester**	**0.71**	**354**	**C23H46O2**
56	**36.74**	**Di-2-Benzothiazole Disulfane**	**0.71**	**332**	**C14H8N2S4**
57	**37.00**	**2-([(2-Ethylhexyl)oxy]Carbonyl) Benzoic acid**	**2.17**	**278**	**C16H22O4**
58	**37.00**	**2-(3,4-Dihydroxyphenyl)-6,8 -Di-β-D-Glucopyronocyl-5, 7-Dihydroxy-4H-1-Benzopyran-4-One**	**2.17**	**610**	**C27H30O16**
59	**37.00**	**1,2-Benzenedicarboxylic acid**	**2.17**	**390**	**C24H38O4**
60	**44.20**	**(3β,24S)-Stigmast-5-en-3-ol**	**1.35**	**414**	**C29H50O**
61	**44.20**	**Ethyl iso-allocholate**	**1.35**	**436**	**C26H44O5**
62	**44.20**	**(3β)-Stigmast-5-en-3-ol**	**1.35**	**414**	**C29H50O**
63	**44.20**	**4-Methyl-(3β,4β)-Cholesta-8,24-dien-3-ol**	**1.35**	**398**	**C28H46O**

The highest percentage compounds with peak percent of 46.89% were (9*Z*,12Z) Octadecadienoyl chloride (also known as Linoleoyl Chloride), well known for anti-microbial activity, (9*E*,12*E*) Octadecadienoyl chloride which showed anti-oxidation properties and (*E*)-9-Octadecenoic acid methyl ester (also known as Methyl Eliadate), with Antioxidant, antiviral and anticancer activities. The second large peak characterized two fatty acid methyl esters with 11.62%, Hexadecanoic acid methyl ester, reported to show cardioprotective and anti-inflammatory effects, the third abundant compound identified in the spectrum was 9,12-octadecadienoicacid methyl ester, reported with antifungal properties. The high ratio compounds and their documented biological activity were listed in Table 2.

Table 2. Reported biological activity of major components of *R. Tripartita* roots Extract

Name	Compound Class	Area%	Biological Activity
(*E*,*E*,*E*) 9-Octadecenoic acid -1,2,3-propanetriyl ester	**Fatty acid ester**	**0.59**	**Anti-spasmodic and immune modulators (5)**

(*Z*)-9-Octadecenoic acid	**Unsaturated fatty acid**	**0.76**	**Reduction of rheumatoid arthritis and a variety of cancers (2)**
(3β,5β)- 2-methylene-Cholestan-3-ol	**Sterol**	**0.82**	**Anti-oxidant (6)**
13-Heptadecyn-1-ol	**Long chain alcohol**	**0.82**	**Anti-inflammatory, anticancer, antimalaria (7)**
***Cis*-Oxiraneundecanoic acid, 3-pentylmethyl ester**	**Fatty acid ester**	**0.91**	**Anti-oxidant (5)**
Hi-Oleic Safflower oil	**Oil**	**1.65**	**Increase health measures (8)**
9,12-Octadecadienoicacid methyl ester	**Fatty acid ester**	**9.92**	**Antifungal (9)**
Hexadecanoic acid, methyl ester (methyl Palmitate)	**Fatty acid ester**	**11.62**	**Cardioprotective effect (10), anti-inflammatory (11)**
14-Methyl-Pentadacanoic acid methyl ester	**Fatty acid ester**	**11.62**	**Antibacterial and antifungal activity (12)**
(*Z*,*Z*)-9,12-Octadecadienoyl chloride (Linoleoyl Chloride)	**Fatty acid halide**	**46.89**	**Antimicrobial agent products (13)**
(9*E*,12*E*)-9,12-Octadecadienoyl Chloride	**Fatty acid halide**	**46.89**	**Antioxidant activity (14)**
• **(*E*)-9-Octadecenoic acid, methyl ester (Methyl eliadate)**	**Fatty acid ester**	**46.89**	**Antioxidant, antiviral, anticancer (15)**
Octadecanoic acid, methyl ester	**Fatty acid ester**	**3.84**	**Antiflammatory (16)**
Cyclopentane tridecanoic acid, methyl ester	**Fatty acid ester**	**3.84**	**Antimicrobial (17)**
2-Undecyl -trans-Cyclopropane pentanoic acid methyl ester	**Carboxylic acid ester**	**3.84**	**Antiviral, antifungal, Anti-HIV, COX-2 inhibitor (18)**
Cyclopropane dodecanoic acid, 2-octyl, methyl ester	**Fatty acid ester**	**3.84**	**Anticancer, antitumor, antiestrogenic, antimicrobaterial (19)**
16-Methyl-Heptadecanoic acid, methyl ester (Margaric acid ester)	**Fatty acid ester**	**3.84**	**Anti-skin cancer (20)**
(*Z*,*Z*),9,12-Octadecadienoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	**Fatty acid ester**	**1.70**	**Anti-inflammatory, Anticancer (21)**

(3β,24S)-Stigmast-5-en-3-ol	**Phytosterol**	**1.35**	**Cholesterol-lowering and antioxidant properties (22)**
Ethyl iso-allocholate	**Steroid derivative**	**1.35**	**Antimicrobial activity (23)**
(3β)-Stigmast-5-en-3-ol	**Steroid**	**1.35**	**Potent anti-diabetic agent in regulating glucose transport (24)**

In addition to the compounds mentioned above, many interesting compounds with low percentage were present in the extract such as Hexadecanoic acid (palmitic acid) and its different esters.

Conclusion

Ethanolic extract of *Rhus Tripartita* roots showed very promising results in terms of the content of bioactive compounds revealed by GC-MS analysis. In this study, organic components from different organic classes with well-known biological activity were identified including, fatty acids, fatty acids esters, alcohols, ketones and sterols. The most abundant components were documented with antibacterial, antiviral, antioxidation, antimicrobial, antifungal, anti-inflammatory and cardioprotective effects. Isolation of individual compounds in a pure state and testing for biological activity would give new horizons drugs discovery.

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