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GC-MS Analysis of European Mistletoe (*Viscum album* L.) Plant Grown at Syrian Coastal Area

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In this study, efforts were made to isolate phtochemicals from European mistletoe (*Viscum album* L.) using hot (Soxhlet) and cold (magnetic rotation) extraction methods. The dried *Viscum album* L. (10 g) were extracted exhaustively by Soxhlet with methanol and dichloroethane solvents. Subsequently, 1 µL of sample was utilized for GC/MS analysis, which exhibited 50 peaks of phytoconstituents in the range of 0.12-22.04%. The highest flavonoids content was determined using quercetin calibration curve having a value of 62.26 mg QE/g dry weight.

Keywords: European mistletoe, Flavonoids, Quercetin, *Viscum album* L.

INTRODUCTION

Globally, people are opting for nature-friendly and natural products, particularly, the use of medicinal plants for treating many diseases [1]. As some parts of Syria have abundance of various types of medicinal plants containing a distinctive proportion of an active substance [2,3], its coastal area is rich in dynamically diverse medicinal plants, especially, *Viscum album* L. (European mistletoe), belonging to the family of Loranthaceae that spontaneously grows in different countries worldwide with stem bouquet, branching cylindrical and full bilateral flower-edge leaves and blooms between the end of winter and spring. The grape fruit plants often contains white sticky plant climbs trees deciduous such as apples and almonds. Compounds such as phospholipids, flavonoids, polysaccharides, choline and acetylcholine are the most crucial components of this plant [4-7].

The glutinous plant or European mistletoe possess several medicinal properties, such as reduced arterial pressure and extensive vascular properties and is useful for the atherosclerosis treatment [8]. Moreover, it contains viscotoxin, thus can strengthen the heart and also lecithin and histamine helps eliminate excess urine from the body, it′s extract is used to treat muscle pain and chronic rheumatism [9,10]. A drug is considered effective against cancerous cells when it contains lecithin [11].

The European mistletoe (*Viscum album* L.) plant is abundant in some parts of Syria and around Syrian coastal areas. This work investigates for the first time to identify the phytocompounds in methanol and dichloroethane extracts of European mistletoe (*Viscum album* L.) by qualitative screening of phytochemicals and to identify each specific compound with their concentrations by gas chromatography-mass spectrum (GC-MS) analysis.

EXPERIMENTAL

The European mistletoe (*Viscum album* L.) plant from the Syrian coastal area was collected during the different growth phases by alternating the periods of time ranging from3 to 4 weeks during the month of August 2018. The plant was identified and authenticated in Al-Andalus University for Medical Sciences and subsequently a voucher specimen was deposited in the herbarium under the collection number AA 0211018/13. The plant was cleaned and the moisture ranging from 10% to 12% was dried to grow the plant under suitable environmental conditions in laboratory. Conditions such as humidity control, temperature and ventilation were changed

to keep the plant intact and prevent from any injury and no pesticides were applied. After the reclamation process, analysis was performed.The extracted plant samples by using two polar solvents, namely methanol and dichloroethane (both procured from Merck with highest purity of 99.9% GR).

Hot reclamation (Soxhlet): A European mistletoe plant (10 g) was placed in a glass cartridge with a length of 9 cm. A 150 mL of dichloroethane/methanol was boiled in bottom glass booths and retrieved in a device connected to a recirculating device with an automatic thermostat. The sample was extracted for 24 h and subsequently evaporated in the rotary evaporator up to 10 mL. Finally, the extract obtained was treated with 0.5 g of anhydrous sodium sulphate for 20 min to remove water content.

Cool extraction (magnetic rotation): A European mistletoe plant (10 g) was placed in a 250 mL flask and then added dichloroethane/methanol (150 mL) and the solution was stirred magnetically by placing a 2 cm magnetic stirrer in it. The solution was rotated with a speed of 500 cycles/min for 24 h and then the sample was filtered through a Büchener funnel. The volatile solvent was then vaporized using the rotary evaporator after the removal of water.

GC-MS analysis: All the four extracts were analyzed quantitatively and qualitatively by using GC/MS with Hewlett Packard-type GC model 5890 detector combined to mass spectrometer HP5970 at a constant temperature and a thermal programming system with a capillary column from silicon oils (DB-5) developed by the natives (phenyl methyl silicone, 5%) and 30 m × 0.32 mm i.d. Liquid stationary phase with a thickness of 0.25 µm and helium (99.99% pure) a gas holder and fast an 1 mL/min was used. Separation was performed in accordance with a thermal program. The samples were injected using a split technology/splitless injector at 250 ºC and 1 µL of each extract was injected to a microsatellite for the analysis. The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification.

Determination of flavonoids: In 250 mL of ethanol:water (1:2) solution, 0.5 g of leaves of European mistletoe plant was placed for 7 days. The sample was then filtered with a 0.45 µm pore filter and stored at 4 ºC. Subsequently, 10 mL of solution was taken and several drops of reagent (3 mL alumina hexachlorite and 2% methanol) were added to obtain a clear colour change from green to yellow. After 10 min of continuous mixing, the solution was filtered using the same filter. The absorption of salinity was measured using a spectrophotometer at 450 nm. In this work, quercetin was used as a standard to quantify the total flavonoid contents of European mistletoe (*Viscum album* L.) plant extract.

RESULTS AND DISCUSSION

Previous studies reported that ethanolic extracts of European mistletoe (*Viscum album* L.) leaves showed, anti-tumor, anti-inflammatory [12] and antioxidant activities [13]. The results represented an important step towards the effective characterization of the secondary class metabolite compounds from this plant using GC-MS analysis.

The GC-MS spectra of the extracts of European mistletoe (*Viscum album* L.) using hot (Soxhlet) and cool (magnetic rotation) extraction methods are shown in Fig. 1. The hot methanolic extract (Soxhlet) showed the presence of only 18

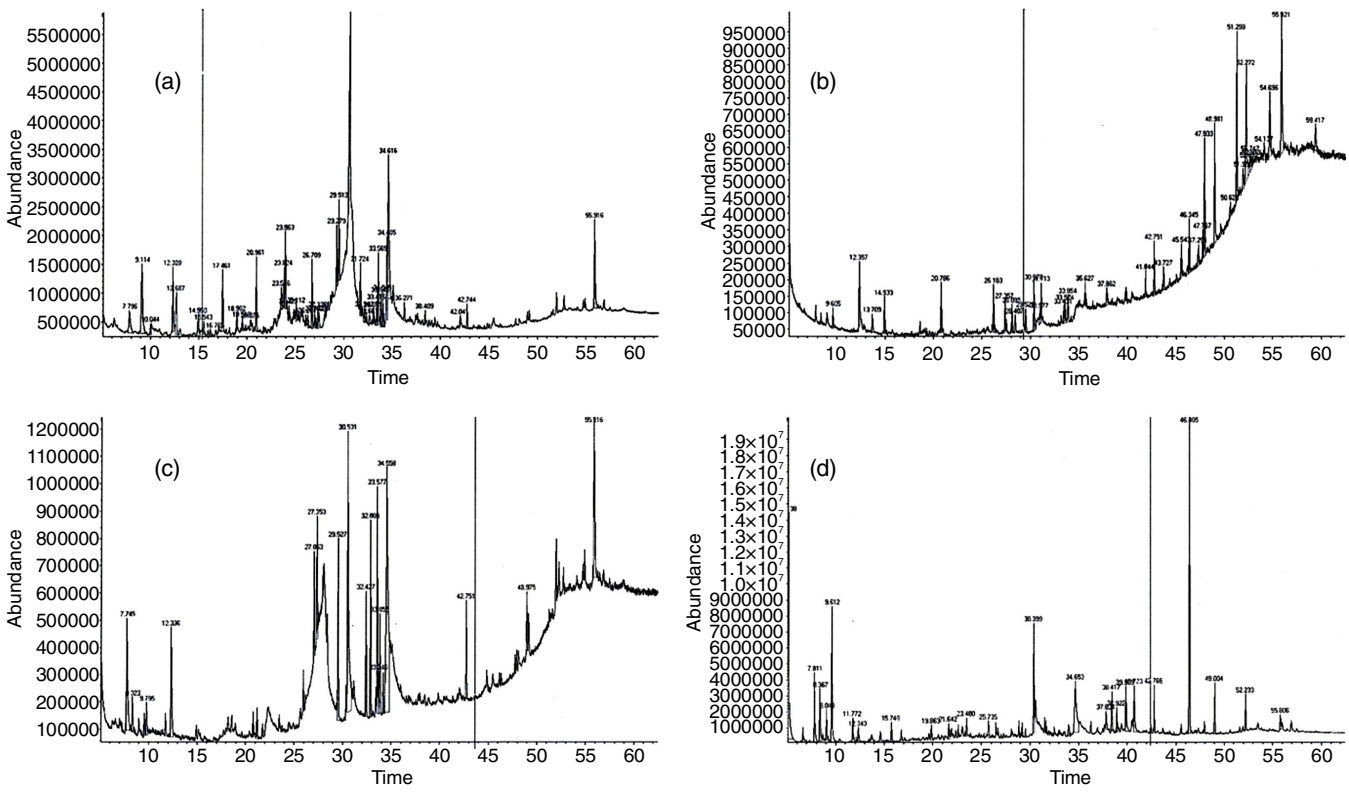

Fig. 1. Chromatogram of (a) methanol-cool extract; (b) methanol-hot extract; (c) dichloroethane-hot and (d) dichloroethane-cool

compounds, while dichloroethane extract exhibited 25 compounds. in case of cool extraction method, both methanolic and dichloroethane extracts showed that only 16 compounds were identified in each. However, using different extraction methods, around 50 compounds were extracted (Table-1).

The percentages of compounds extracted by hot and cool extraction methods were ranged between 0.12-22.04%. The highest percentage was for the palmitic acid which was 22.04% followed by eucalyptol or cineol (1.89%). The next major acid was olic acid, which consisted of 11.30% in the sample followed by methyl acetate acetadicanoic acid (9.03 %). However, total flavonoids constitute around 62.26% in European mistletoe (*Viscum album* L.) plant indicated the presence of highly antioxidant properties as comparison to other reports [13,14].

TABLE-1
GC-MS ANALYSIS DATA OF PHYTOCHEMICALS IN EUROPEAN MISTLETOE (*Viscum album* L.) USING HOT (SOXHLET) AND COOL (MAGNETIC ROTATION) EXTRACTION METHODS

S. No.	Name of composition	m.f.	Hot extraction method (Soxhlet)		Cold extraction method (Magnetic rotation)	
			Dichloro-ethane (%)	Methanol (%)	Dichloro-ethane (%)	Methanol (%)
1	*N*-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	4.20	18.12	–	22.04
2	Eucalyptol	$C_{10}H_{14}O$	1.89	–	–	–
3	Oleic acid	$C_{18}H_{34}O_2$	7.95	–	–	11.30
4	Methyl *cis*-7-octadecenoate	$C_{19}H_{38}O_2$	–	–	–	9.03
5	Methyl palmitate	$C_{17}H_{34}O_2$	0.34	2.45	1.19	8.86
6	1-Tricosene	$C_{23}H_{46}$	–	–	7.76	–
7	3-[5,5,6-Trimethylbicyclo[2.2.1]hept-2-ene]cyclohexan-e-one	$C_{10}H_{16}O$	6.19	–	–	–
8	Polyglycol dialkyl ether	R_1O-(CH_2CH_2)	–	–	6.04	–
9	Biosol	$C_{10}H_{14}O$	–	–	5.10	2.43
10	22*R*,23*R*-Dihydrostigmasterol	$C_{29}H_{50}O$	–	4.04	2.46	–
11	γ-Stigmasaterol	$C_{29}H_{48}O$	–	–	–	4.03
12	9-Octadecene	$C_{18}H_{36}$	–	3.57	–	–
13	1,7,7-Trimethylbicyclo[2.2.1]hepten-2-ene	$C_{12}H_{20}O$	3.35	–	–	3.20
14	2,3-Dihydro-1,1,3-trimethyl-3-phenyl-1*H*-indene	$C_{16}H_{14}$	0.50	2.92	–	–
15	Borneol	$C_{10}H_{18}O$	2.82	–	–	–
16	1-Octadecene	$C_{18}H_{36}$	–	–	2.80	–
17	*p*-Thymol	$C_{10}H_{14}O$	0.38	2.64	–	–
18	*cis*-11-Tetradecen-1-ol	$C_{14}H_{28}$	0.37	2.60	–	–
19	Phytol	$C_{20}H_{40}O$	–	0.81	1.64	2.46
20	3-Methyl-4-isopropylphenol	$C_{10}H_{14}O$	–	–	–	2.43
21	Cyclododecanol	$C_{12}H_{24}O$	–	2.36	–	–
22	2-Tetradecene	$C_{14}H_{28}$	–	–	2.27	–
23	1-Tetradecene	$C_6H_{14}O$	–	–	1.93	0.33
24	Nonacosan-10-one	$C_{14}H_{28}O$	2.17	–	–	–
25	Tetradecanal	$C_{16}H_{32}O$	0.88	2.08	–	–
26	2-Tridecyloxirane	$C_{16}H_{32}O$	–	2.08	–	–
27	1,19-Eicosadiene	$C_{20}H_{38}$	0.49	0.49	1.81	–
28	2-([(2-Ethylhexyl)oxy]carbonyl)benzoic acid	$C_{11}H_{12}O_4$	1.78	–	–	–
29	(1*S*)-4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-one	$C_{10}H_{14}O_2$	–	–	1.37	–
30	Hexacosanol	$C_{16}H_{34}O$	–	–	–	1.28
31	4-Hydroxy-α,α,4-trimethylcyclohexanemethanol	$C_{10}H_{18}O$	1.27	–	–	–
32	Pyridine	C_5H_5N	–	–	1.25	–
33	Octadecanal	$C_{18}H_{38}$	0.85	1.21	-	–
34	Cyclotetradecane	$C_{14}H_{28}$	–	–	1.19	–
35	1-Eicosene	$C_{20}H_{40}O$	–	–	1.15	–
36	(Z,Z)-9,12-Octadecanoic acid	$C_{18}H_{32}O_2$	–	–	1.04	0.43
37	4-(1,1,3,3-Tetramethylbutyl)phenol	$C_{14}H_{22}O$	–	–	–	0.56
38	Methyl octadecanoate	$C_{11}H_{22}O_2$	–	1.95	–	0.76
39	Methyl 16-methylheptadecanoate	$C_{19}H_{38}O_2$	–	0.31	–	0.52
40	Methyl *trans*-9-octadecenoate	$C_{18}H_{20}$	0.55	0.46	–	–
41	Di-*n*-Octylphthalate	$C_{24}H_{38}O_4$	–	0.66	–	–
42	Methyl *trans*-9-octadecenoate	$C_{19}H_{36}O_2$	–	0.55	–	–
43	3,7-Dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	0.38	–	–	–
44	1-Ethyl-1,3,3-trimethylcyclohexane	$C_{12}H_{25}$	0.37	–	–	–
45	4-Isopropyl-2-methylphenol	$C_{10}H_{14}O$	0.38	–	–	–
46	1,2-Epoxyoctadecane Hexadecyl oxirane	$C_{18}H_{36}O$	0.71	–	–	–
47	Hexadecanal	$C_{16}H_{32}O_2$	0.62	–	–	–
48	13-Isopropylpodocarpen-12-ol-20-al	$C_{27}H_{54}O_2$	0.12	–	–	–
49	Olean-12-ene	$C_{30}H_{50}$	0.90	–	–	–
50	α-Amyrenol	$C_{30}H_{50}O$	0.68	–	–	–

Conclusion

The GC-MS results indicated that European mistletoe (*Viscum album* L.) plant grown at Syrian costal areas contains 50 phytochemcials ranged between 0.12-22.04% when extracted with two different extraction methods *viz*. hot (Soxhlet) and cold extraction methods using methanol and dichloroethane as extracting solvents. GC-MS analysis confirms the presence of the various secondary metabolite compounds detected by the qualitative procedures. The current study suggested that different extraction methods and different solvents play an important role in the extraction of important bioactive groups.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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