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# Chemical components and antibacterial activity of *Gundelia tournefortii* (L.) Compositae/Asteraceae (Iraq, Kurdistan Region, Sulaymaniyah, Penjwin area, "Kokhalan")

**Arsalan M. Mahmood*, Alhan K. Sallo and Mahmood Ali Hasan**

Faculty of Science, Chemistry Department, University of Duhok, Kurdistan Region, Iraq

*E-mail* : Arsalanch23@yahoo.com

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**Abstract : Ethyl acetate and hexane extracts of *Gundelia tournefortii* (L.) (Compositae/Asteraceae family) consist of the following components which are determined by gas chromatography-mass spectrometry (GC-MS). Both extracts contain 4 expected compounds. Hexane extract involves three components : methyl 14-methylpentadecanoate (27.43%), linoleic acid, methyl ester (42.32%), linolenic acid, methyl ester (29.48%). While one component was found in ethyl acetate extract : spiropentane, butyl (55.91%). The antibacterial activities of ethyl acetate and hexane extracts against pathogenic bacteria (*Staphylococcus aureus, Bacillas subtilis, Escherichia coli, Klebsilla pneumonie, Pseudomonas aeruginosa*) were measured using well diffusion methods. Considerable differences in the antibacterial activity of plant extracts were noticed depending on the used bacterial strain or the type and concentration of plant extracts.**

**Keywords : *Gundelia tournefortii*, Compositae, chemical components.**

## Introduction

The species *tournefortii* belongs to the *Gundelia* family (Compositae/Asteraceae family) (Fig. 1) which looks like a thistle, spiny plant. They occur in desert areas of Iraq, Syria, Palestine, Iran, Armenia, Anatolia[1], Western Asia, Cyprus, Egypt, Israel, Jordan, Turkey, Azerbaijan and Turkmenistan[2].

After the autumn rains the plant becomes a rosette and bolts during February-April. Each branch ends with a compound spiny ovoid head 4–8 cm in diameter reaching a height of 50 cm[3].

It is known that the flower itself, leaves, seeds and stems are also used as food sources[2], medicine, folk medicine to prepare traditional recipes such as diarrhea, mumps and diabetes. It is even mentioned in local folkloric songs as well as proverbs[1,4]. In Eastern Anatolian folk medicine dry seeds of *G. tournefortii* were used for healing the vitiligo disease[2]. In Jordanian *G. tournifortii* is known in for the treatment of chest pain and heart stroke. In Turkey, this plant is used in folk medicine to remove water from patients having spleenomegaly[3].



**Fig. 1.** *Gundelia tournefortii* (L.) (Compositae/Asteraceae family).

Both the aerial parts and seeds of *G. tournefortii* have antioxidant potential[5]. Chemical constituents have different types of compounds called : (1) scopoletin, (2) isoscopoletin, (3) esculin, (4) β-sitosterol and (5) stigmasterol. Scopoletin has a similar mass spectrum for 7-hydroxy-6-methoxy coumarin[3]. Major phenolics contained gallic acid and quercetin[6]. First discoveries have been made on the GC-MS analysis of *G. tournifortii* grown in Kurdistan.

## Results and discussion

Seven expected compounds in hexane and ethyl acetate extracts of *Gundelia tournefortii* were determined

and identified the structures names of only four expected compounds including their molecular formula and molecular weight (Table 1). The GC-MS chromatograms of *n*-hexane and ethyl acetate extracts shown in Figs. 2 and 3. In hexane extract three expected components were identified (Table 1) : methyl 14-methylpentadecanoate (27.43%), linoleic acid, methyl ester (42.32%), linolenic acid, methyl ester (29.48%). The major chemical constituent in the hexane extract at $R_t$ 35.462 was shown fragment peaks at *m/z* 294 (M), 263, 164, 150, 135, 123, 67, 41. The mass spectrum of linoleic acid, methyl ester is similar to Fig. 4[7]. The fragment ions were formed as *m/z* 263 (M-$CH_3O$), 164 (M-$C_{10}H_{18}O$), 150 (M-$C_{11}H_{20}O$), 135 (M-$C_{11}H_{20}O_2$), 123 (M-$C_{12}H_{20}O_2$) and the main peak 67 (M-$C_{16}H_{28}O_2$).

The second major compound in $R_t$ 35.591 have fragment peaks at *m/z* 292 (M), 149, 135, 121, 108, 79 and the spectrum of linolenic acid, methyl ester was the same Fig. 5[7]. The main fragment was formed as *m/z* 149 (M-$C_8H_{15}O_2$), 135 (M-$C_9H_{17}O_2$), 121 (M-$C_{10}H_{19}O_2$), 108 (M-$C_{11}H_{20}O_2$) and the standard peak 79 (M-$C_{14}H_{23}O_2$).

The $R_t$ of third major compound equal to 32.267 and fragment peaks shown in *m/z* 270 (M), 227, 213, 129, 74. The Fig. 6 was the same as the mass spectrum of methyl 14-methylpentadecanoate[8]. The major fragment ions formed as *m/z* 227 (M-$C_2H_3O$), 213 (M-$C_3H_5O$), 129 (M-$C_9H_{17}O$) and the standard peak 74 (M-$C_{13}H_{24}O$).

One compound was found in the ethyl acetate extract spiropentane, butyl (55.91%) (Table1). The main chemical component at $R_t$ (35.474) the ethyl acetate extract

**Table 1.** The major components of hexane and ethyl acetate extract in *Gundelia tournefortii*

| Compd. | $R_t$ | Molecular formula | Mol. wt. | Peak area (%) | Structures of expected compounds | Name |
|---|---|---|---|---|---|---|
| **1** | 35.462 | $C_{19}H_{34}O_2$ | 294 | 42.32 | | Linoleic acid, methyl ester |
| **2** | 35.591 | $C_{19}H_{32}O_2$ | 292 | 29.48 | | Linolenic acid, methyl ester |
| **3** | 32.267 | $C_{17}H_{34}O_2$ | 270 | 27.43 | | Methyl 14-methylpentadecanoate |
| **4** | 35.474 | $C_9H_{16}$ | 124 | 55.91 | | Spiropentane, butyl |

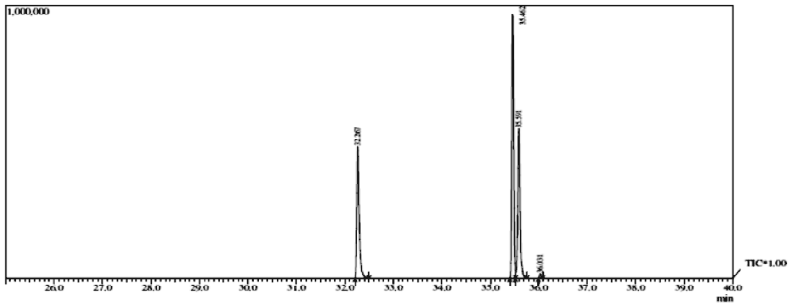



**Fig. 2.** GC-MS chromatogram of the hexane extract of *Gundelia tournefortii*.

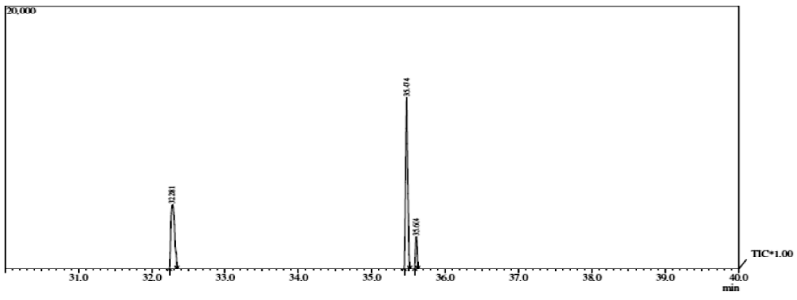



**Fig. 3**. GC-MS chromatogram of the ethyl acetate extract of *Gundelia tournefortii*.

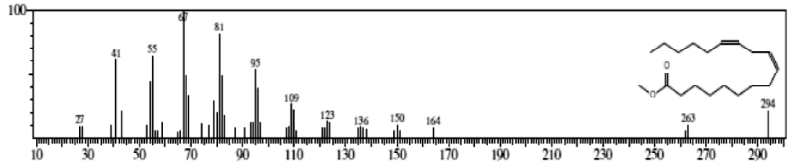


**Fig. 4**. Mass spectrum of linoleic acid, methyl ester.

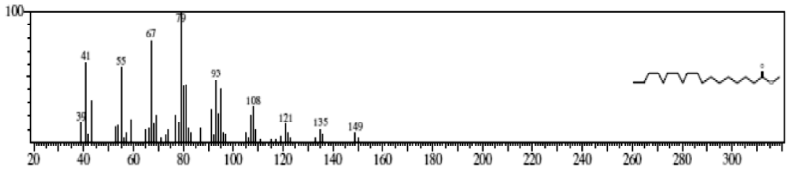


**Fig. 5**. Mass spectrum of linolenic acid, methyl ester.

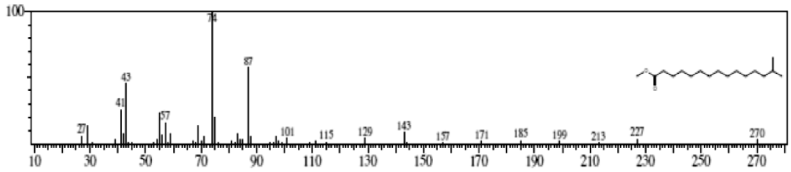


**Fig. 6**. Mass spectrum of methyl 14-methylpentadecanoate.

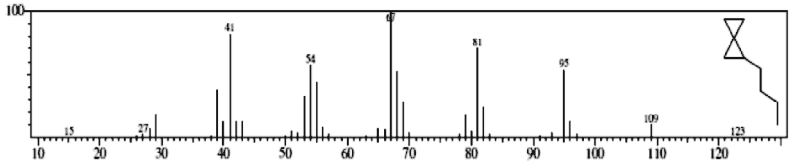


**Fig. 7**. Mass spectrum of spiropentane, butyl.

show fragment peaks at *m/z* 123 ($M^+$), 109, 95, 81, 67. The spectrum of spiropentane, butyl was the same Fig. 7[9]. And major fragment ions result at *m/z* 109 ($M^+$-$CH_2$), 95 ($M^+$-$C_2H_4$), 81 ($M^+$-$C_3H_6$), the standard peak was 67 ($M^+$-$C_4H_8$).

Table 2 shows the results for antibacterial activity of hexane and ethyl acetate extracts indicating considerable differences in the antibacterial activities among extracts depending on the bacterial strain and the concentration of extracts used. The results in this study emphasized on the Gram-positive bacteria were more susceptible than the Gram-negative bacteria like *P. aeruginosa* which show more resistance than *B. subtilis* and *S. aureus*. The reason would be that libopolysaccharide (LPS) Gram-negative bacteria layer on the surface has high hydrophobicity and behaves like a strong barrier against hydrophobic molecules[10]. However, Gram-positive bacteria can enter the cell wall easier than the Gram-negative bacteria since the cell wall of the Gram-positive bacteria contains peptidoglycan and lacks an outer membrane[11].

*Material and methods* :

*Plant's material* :

*Gundelia tournefortii* was collected in April 2012 from the area of Penjwin in Kokhalan, Sulaymaniyah, Kurdistan, Iraq. Dr. Salim Shahbaz, Faculty of Agriculture, University of Duhok identified it.

*Chemicals and equipments* :

(a) Chemicals : Methanol, dichloromethane, hexane and ethyl acetate obtained from (UNI-Chem, China) and distilled water.

**Table 2.** Antibacterial activity (inhibition zone/mm) of *n*-hexane and ethyl acetate extracts and standardized antibiotic (Tetracycline and Kanamycine) against Gram-positive and Gram-negative pathogenic bacteria

| Extract | ppm | Test organisms | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* ($G^+$) | *B. subtilis* ($G^+$) | *E. coli* ($G^-$) | *P. aeruginosa* ($G^-$) | *K. pneumonia* ($G^-$) |
| Hexane | 50 | 8 mm | 10 mm | 7 mm | 8 mm | R |
| | 100 | 10 mm | 10 mm | 10 mm | 9 mm | 7 mm |
| | 200 | 10 mm | 11 mm | 10 mm | 9 mm | 7 mm |
| | 500 | 11 mm | 11 mm | 10 mm | 10 mm | 8 |
| Ethyl acetate | 50 | 13 mm | 10 mm | 10 mm | 9 mm | 7 mm |
| | 100 | 13 mm | 11 mm | 10 mm | 9 mm | 8 mm |
| | 200 | 14 mm | 12 mm | 11 mm | 10 mm | 8 mm |
| | 500 | 14 mm | 12 mm | 11 mm | 10 mm | 9 |
| Tetracycline | 10 μg | 10 mm | 5 mm | R | R | 12 |
| Kanamycine | 10 μg | R | R | R | 5 | 10 |

R = Resistant, mm = Milimeter, $G^+$ = Gram-positive, $G^-$ = Gram-negative.

(b) Pathogenic bacteria used in : *Staphylococcus aureus, Bacillas subtilis, Escherichia coli, Klebsilla pneumonie, Pseudomonas aeruginosa* (Azadi Hospital Laboratory).

(c) Glassware of laboratory, Vacuum Rotary Evaporator (VRE) distillation apparatus.

(d) GC-MS [Shimadzu QP2010], Department of Chemistry, Faculty of Science, University of Malaya UM, Kuala Lumpur, Malaysia.

*Extraction method* :

The 10 kg of fresh plant was weighed, were cleaned, and the bulbs directly is extract at a mangle by a mechanical grinder. The remained material after mangling were put into the stoppard flask fully immersed with methanol. In a period of seven days the flask was shaken from time to time and filtered; the filtration had evaporated by VRE. The methanol extract is 15 g and divided individually with hexane, dichloromethane (DCM) and ethyl acetate to get four extracts; (0.5 g) of hexane, (0.1 g) DCM, (0.4 g) ethyl acetate and finally the residue (2.2 g) of methanol extracts. Only *n*-hexane and ethyl acetate extracts had a result in gas chromatography-mass spectroscopy technique.

*Analysis by gas chromatography-mass spectrometric* :

*Software of gas chromatograph* : The length of capillary column : 30.0 m, diameter : 0.25 mm and film thickness : 0.25. The column oven temperature was 50 °C and injector temperature to 250 °C. Oven temperature was programmed on 5 °C/min. The carrier gas : helium 99.9995% purity at 1.51 ml/min. The injected sample (1 μL) was about 10 : 1. The running time included 30 min while the mass spectra were taken with scan range of 40–1000 *m/z* of 0.5 s intervals at 70 eV ionization. The percentage areas of the components were calculated by comparing to the total area.

*Antibacterial activity* :

Antibacterial activity of all prepared (hexane and ethyl acetate extract) were tested against five isolates of Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli*, *K. pneumonie* and *P. aeruginosa*) pathogenic bacteria. Circular disc of 6 mm diameter were made from the Whatman number 1 filter paper. Discs were impregnated with equal volume (50 μL) of each complex. The discs were aseptically placed over plates of Muller Hinton agar seeded with each of the test pathogens. The antibacterial assay plates were incubated at 36 °C for one day and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 10 mm were considered as having low antibacterial activity. Diameters between 10 and 14 mm were considered moderately active and these with more than 16 mm were considered highly as being active[12]. Antibiotics were used Tetracycline and Kanamycine (Table 2). The standard discs of both antibiotics Tetracycline and Kanamycin (10 μg per disc) functioned as positive antibacterial control. The diameter of inhibition around each of the discs was taken as a measure of the antibacterial activity. The di-

ameters of the zones of inhibition by the samples were then compared with the standard antibiotic disc used. Four different concentrations were prepared for ethyl acetate and hexane plant extract (50, 100, 200 and 500 ppm).

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