A comparative study between the two Euphorbia L. species by used chemical and antioxidant evidence

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Abstract

Background : E.hypericifolia and E.prostrata of the genus Euphorbia are one of the important medicinal plants. The current study was designed to conduct an analysis of the chemical compounds in the leaves and to study the antioxidant potential of the leaves extract as a comparative study . Material and Methods : The leaves extract was prepared using three different solvents, then the antioxidant potential was tested by measuring the radical scavenging activity of DPPH. Then the chemical analysis was carried out using gas chromatography - mass spectrometry of the methanolic extract . Results: The chemical study revealed a number of active chemical compounds and The analysis results showed a high percentage of fatty acids, alcohols, tannins and other compounds that have an effective antifungal and antibacterial. he results confirmed the antioxidant activity, as the highest rate of scavenging activity was in the methanolic extract , and the lowest efficacy was in the ethyl acetate and aqueous extract of both species , and the highest scavenging activity was in the leaves extract of E. prostrata compared to E. hypericifolia. Comparing the IC50 value of the plant extract with Ascorbic acid, the results showed that the leaf extract possesses high efficacy as an antioxidant . conclusions. The results revealed the importance of the chemical study to detect the active chemical compounds and as a taxonomic aspect to separate the two species. As well as the importance of the alcoholic extract of E. prostrata as a powerful antioxidant.

Keywords: *Euphorbia, Antioxidant, DPPH, Phytochemical.*

1. INTRODUCTION

The Euphorbiaceae family is the fourth largest family of flowering plants, comprising a large number of genera and species. The members of Euphorbiaceae family are common in arid and semiarid environments, mainly in tropical and subtropical regions of Africa and America. .The family has approximately 283 genera and 7,300 species (Thakur et.al, 2012) . In Iraq, seven genera were recorded for the family within the Iraqi flora, including the genus Euphorbia L. (Al-Musawi,1987) .

Numerous characteristics were recorded for members of the Euphorbiaceae family , especially the genus Euphorbia L, including the ability to induce dermatitis (Seigler ,1994)

, an important source of drugs and toxins. (Devappa et.al, 2010) ; (Ramalho et.al, 2017) .In addition to the latex milk substance that is frequently present in the members of the Euphorbiaceae family, as (Vasas et.al,2012) confirmed the presence of natural products with high biological effectiveness in latex rubber milk. It has also been recorded that some species have been used as a tonic, narcotic, anti asthmatic, effective against dysentery, diarrhea and colic, especially amebiasis. (Mishra and Parida,2020) . Also, some plant extracts from the Euphorbiaceae family were used as medicines, including the drug Euphorbium (Resiniferatoxin), which is made from the plant milk of the species E. resinifera, known by the trade name (Complexe Leh ning Euphorbium N88). It was used as a nasal spray or a compound against viral infections, sinusitis, and chronic nasal secretions, as well. For the treatment of dry and inflamed mucous membranes lining the nose, as well as symptoms of the flu. (Bijekar and Gayatri , 2014) .

The genus Euphorbia L. is the second largest genus of flowering plants, and the largest genus in the Euphorbiaceae family (Frodin , 2004). There are 2100 species recorded for the genus spread in both hot and tropical regions. In Iraq: about 40 species were recorded. (Al-Musawi , 1987) . The genus Euphorbia L has been described in many scientific studies as a medicinal plant and also a plant that contains a toxic substance (AL-Rawi and Chakravatry, 1964) . Several studies have indicated the biological activity of the genus Euphorbia L as an antioxidant. (Rauf et.al, 2012) ; (Prabha et.al, 2014) . Also, studies have been conducted showing the antibacterial and antifungal effect of different species of the genus Euphorbia L. , including E. prostrata and E. hypericifolia . Since ancient times, this plant has been traditionally used to treat many ailments . The active chemical conistituents in the two species chiefly are flavonoids, phenolic acid, fatty acids, and tannins. (Shih-Huei and Ming-Jou,2004) mentioned in an article on E.hypericifolia L. that it has several biological activities, including antipyretic, astringent, emmenagogue, laxative and anesthetic, also as a diaphoretic and in traditional Chinese medicine. also , (Mbayo et.al, 2016) indicated that this species has an effective use in treating Gonorrhoea, by drinking a decoction of the whole plant or leaves. It also has a good role in treating wounds. Also ,(Rauf et.al, 2012) showed that the maximum antioxidant capacity of E.prostrata strongly supports the use of this plant in many foods for several conditions such as fever, blood purifier,anti-inflammatory and even as an antibiotic. Thus, the current study aims to investigate the chemical nature of the bioactive components and to evaluate the activity as antioxidants of the methanolic extract of leaves of two species E.prostrata and E.hypericifolia.

2. MATERIALS AND METHODS:

2.1: Collection and preparation of plant matter

The plants were collected from different parts of the country . The species was diagnosed by plant taxonomist in Department of Biology / College of Science / University of Babylon , Iraq. The samples were collected for the period between March - September 2022. Then , The leaves were cleaned, washed and dried under shade at room temperature for week .

2.2: Preparation of the Plant extract :

A: methanolic extract :

The extraction process was carried out according to the method of (Akowauh et.al, 2004) and (Bazzano and Serdula , 2003) as follows:

1: The dry leaves of the two species E.prostrata and E.hypericifolia were grinded by using an electric mill to obtain the powder.

2: 80g of each plant sample were placed in 1L.volumetric flasks in a hydroalcoholic mixture consisting of (water, methanol) (400 ml - 400 ml) with the opening of the container tightly closed with aluminum foil to avoid evaporation of the mixture, and to prevent air oxidation .

3: The flasks were placed in the rocking water bath at 37°C for half an hour at high speed.

4: The suspension was filtered by guise and the filtered liquid was concentrated to dryness in oven at 45 °C for 24 hours. The dried concentrated material was milled by using electric mill and finl powder was sterilized by UV equipment for 20 min.

B: Ethyl acetate and Hot water extract :

The extract was prepared in the same way as the hydroalcoholic extract by using the solvent ethyl acetate at a concentration (800 ml).

2.3: Evaluation of antioxidant activity by DPPH radical scavenging method :

DPPH (1, 1-diphenyl 1-2-picryl-hydrazyl) is a stable free radical because of the delocalization of spare electron with maximum absorption at about 517 nm. This delocalization gives rise to the deep violate color, the redact form is result from mixing the DPPH solution with that of a substance which can donate a hydrogen atom (Ghobashy et.al, 2021) . The test was carried out according to the method (Williams et.al, 1995) , With some minor modifications.

Free radical scavenging activity of different extracts (50% methanolic aqueous , Ethyl actate and Hot water) of plants were measured by (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (3.12, 6.25, 12.5, 25, 50, 100 and 200 µg/ml). The mixture was shaken vigorously, then the samples were incubated at 37ºC for 30 minutes in dark place. then, absorbance was measured at a wave length 517 nm. by using spectrophotometer. Reference standard compound being used was ascorbic acid . The experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using following equation:

$$\text{DPPH scavenging effect (\%) or Percent inhibition} = A0\text{-}A\,1 / A0 \times 100$$

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample .

Later, an inhibitory concentration of 50% of the activity of DPPH (IC50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical) was calculated for each extract based on the equation that determines the percentage of inhibition and then compared with that of ascorbic acid. (Yingkum et.al, 2002). The positive control (ascorbic acid) was prepared in the same way as the plant extract, and in the same concentrations. It was used for comparison.

- The DPPH test was conducted by using a plastic cuvette tube (volume of 3 ml).

2.4: Chemical analysis by Gas Chromatography - Mass Spectrum

Gas chromatography-mass spectrometry (GC-MS) is one of the modern techniques used to determine the chemical components and their properties present in the mixture, knowing the chemical composition and molecular weight through the use of an appropriate method of operation that combines gas chromatography and mass spectrometry detector for different materials within a test sample. (Zhu et.al, 2021).

GC-MASS analysis procedures :

- An extract was prepared by dissolving 1gm of E.prostrata and E.hypericifolia leaves in 3ml of methanol.
- The mixture was mixed with a vortex for 3 min. under 3000 rpm, then filtered.
- A filtration process was carried out using milpore 0.20 mm diameter .
- It was diluted by 1/10 and 1.5 ml was taken from it and placed in vials glass .
- The examination was carried out using an Agilent 7890 A gas chromatograph and a 5975 C mass spectrometer under the control of (70) EV. The capillary column was HP-5MS (methyl silicone), with a length of 30 m, an inner diameter of 250 µm, and a thickness of films 0.25 µm.
- Helium gas was used as a carrier gas at a constant flow rate of 2ml per minute with an injection volume of µl with an injector temperature of 250°C .
- After that, the survey was done for 18.5 min., with an initial temperature of the oven (50°C) for one minute, then it increased to reach 240 degrees, and stabilized at the last 5 minutes. The rate of temperature increase inside the oven is 15.2 °C for 12.5 min..
- The GC-MAS peaks were detected based on total ion chromatography (TIC) and mass chromatography with compounds identified using the mass spectrometry library of the National Institute of Standards and Technology. (Hashim et.al, 2014) (NIST).

3. THE RESULTS

1: GC-MAS analysis

GC-MAS analysis was conducted on the methanolic extract of leaves of E.prostrata and E.hypericifolia as a comparative study, and the results of the analysis were able to identify a number of chemical compounds. Figure (8 and 9).

The identified chemical constituents , biological activity of the identified chemical constituents, retention time, chemical structure, molecular weight, and structural formula are recorded in Table (3). The results of the current study showed a difference in the numbers and types of chemical compounds identified from the methanolic-aqueous extract of the leaves of the two species E.prostrata and E.hypericifolia. In the leaves of E.prostrata,a number of fatty acids were identified, among them (12-Bromododecanoic acid) ; Stearic acid ; Capric acid ; Tridecanoic acid ; Hexadecanoic acid ; Myristic acid ; 2-Heptadecanol ; Methyl-2-norcaranone . In addition to a number of effective chemical compounds such as D-Glucose, 4-O-alpha. -D-glucopyranosyl- ; Benzoic acid ; as well as some phenolic compounds, including 1,2,3-Benzenetriol; and alkaloid compounds, including 1H-1,2,4-triazol-5-amine and pyrazine trimethyl; Maleinimide. Also, the results of the analysis recorded some of the terpen compounds, including Neophytadiene . and other compounds such as 2-Furancarboxaldehyde 5-hydroxymethyl .

As for the extract of E. hypericifolia leaves , approximately (13) compounds were recorded. Some of these compounds are fatty acids, such as the compound; Hexadecanoic acid , methyl ester , PALMITC ACID ; Heptacosanoic acid, methyl ester; Propanoic acid, 3-chloro-, ester. Also, including terpene compounds such as Camphene, and nitrogenous compounds such as Benzene , 2-methyl-1,3-dinitro and 3-(5-Bromo-3- nitro -1H-1,2,4-triazol-1-yl)-6,8-dioxabicyclo[3.2.1]octan-4-one, some of which belong to the ketone group as alpha-Bromo-p-methoxycetophenone.

2: Antioxidant activity

Antioxidants are substances capable of inhibiting oxidative processes and thus block

the chemical reaction that transfers electrons or hydrogen to an oxidizing agent such as glutathione (Devasagayam et.al, 2004). As a comparative study, during the current study, the antioxidant activity was determined using the DPPH method, as different solvents (Methanol, Ethyl acetate and Hot water) were used to make a plant extract of E.prostrata and E.hypericifolia leaves . Also, different concentrations were taken (200, 100, 50, 25, 12.5, 6.25, 3.12) (µg/ml) for each solvent. Table (1 and 2). The results showed that the highest rate of scavenging activity was in the methanolic - aqueous extract, and the lowest rate of activity was in the (ethyl acetate and the aqueous) extract for both species under study. In addition, the study recorded the highest rate of scavenging activity in the methanolic-aqueous extract of E. prostrata , which was (94.02%, 86.14%, 82.08%, 66.95%, 53.65%, 44.59% and 41.16%), while the activity rate was recorded in E. .hypericifolia (79.84, 61.80, 53.02, 46.98, 35.71, 31.29, 24.34) at concentrations µg/ml (200, 100, 50, 25, 12.5, 6.25, 3.12) for both species . the methanolic- aqueous extract of E. prostata has high antioxidant activity compared to that of ascorbic acid , while E.hypericifolia showed lower effectiveness compared to that of Ascorbic acid except for the highest concentration .Also, The results of the study confirmed that the methanolic-aqueous extract of E.prostrata has strong antioxidant activity compared to E. hypericifolia .

Plant extract	**Scavenging activity (%)**						
	(methanolic)		**Ethyl acetate**		**Hot water**		**Ascorbic acid**
Concen.(µg/ml)	***E.p***	***E.h***	***E.p***	***E.h***	***E.p***	***E.h***	
0.0	**0**	0	0	0	0	0	0.0
200	94.02	79.84	52.22	68.78	72.16	69.85	77.25
100	86.14	61.80	34.81	46.35	41.25	53.93	74.39
50	82.08	53.02	31.01	39.75	24.63	34.68	72.49
25	66.95	46.98	26.70	38.27	20.90	22.29	64.34
12.5	53.65	35.71	24.90	34.92	14.20	21.41	54.50
6.25	44.59	31.29	23.70	31.57	17.84	20.46	50.79
3.12	41.16	24.34	23.46	28.84	12.45	17.09	38.94

E.p = Euphorbia prostrata E.h = Euphorbia hypericifolia.

Table (2): Antioxidant Activity of plant extracts against Free-Radicals. (Phongpaichit et.al, 2007)

Plant	**Solvent**	**IC50 (µg/ml)**	**Mark**
E. prostrata	Methanolic	23.45	Strong
E.hypericifolia	Methanolic	77.31	Moderate
E. prostrata	Ethyl acetate	179.72	Weak
E.hypericifolia	Ethyl acetate	110.14	Weak
E. prostrata	Water	127.84	Weak

E.hypericifolia	Water	115.63	Weak
Ascorbic acid		31.97	Strong

Figure (1): The antioxidant activity of Ascorbic acid

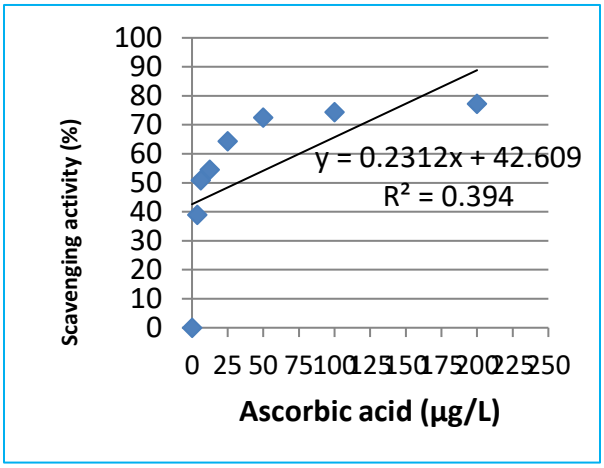


Figure (2): The antioxidant activity of the methanolic extract of E.prostrata leaves

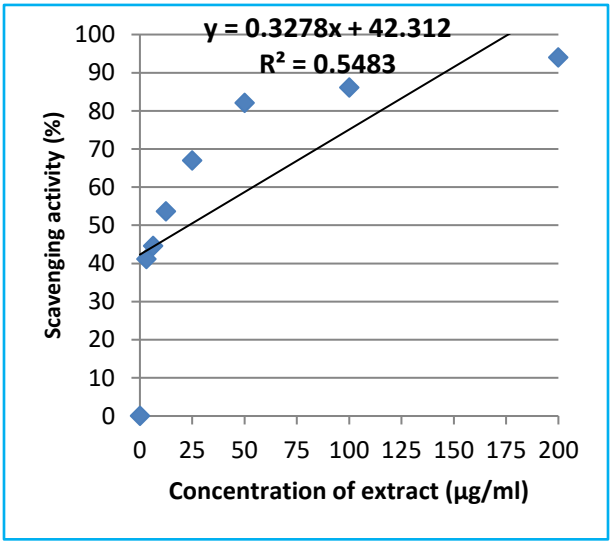


Figure (3): The antioxidant activity of the methanolic extract of E. hypericifolia leaves

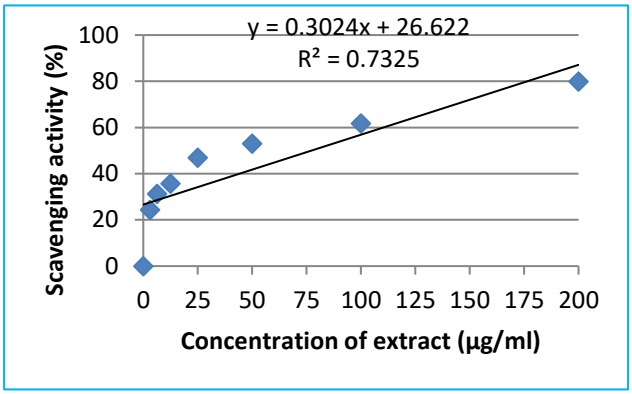


Figure (4): The antioxidant activity of ethyl acetate extract of E.prostrata leaves.

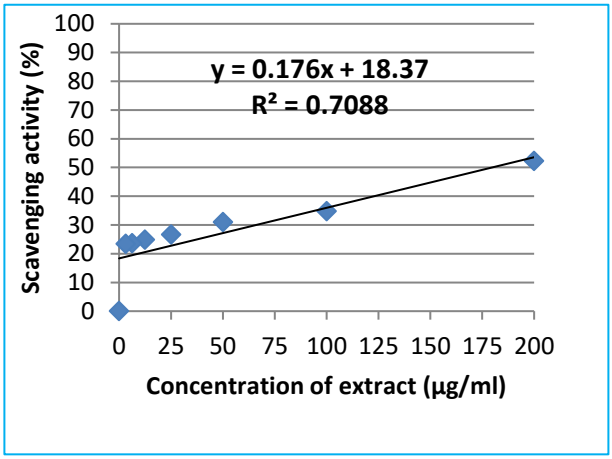


Figure (5): Antioxidant activity of ethyl acetate extract of E.hypericifolia leaves.

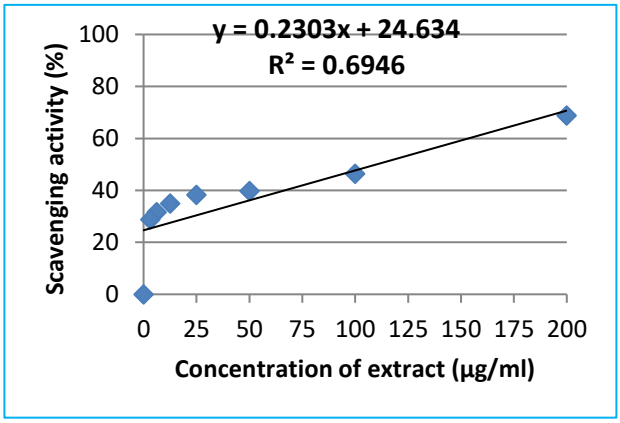


Figure (6) Antioxidant activity of hot water extract of E. prostrata leaves

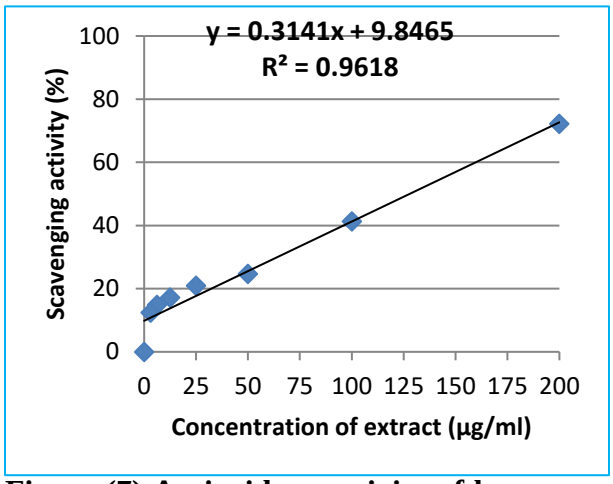


Figure (7) Antioxidant activity of hot water extract of E. hypericifolia leaves

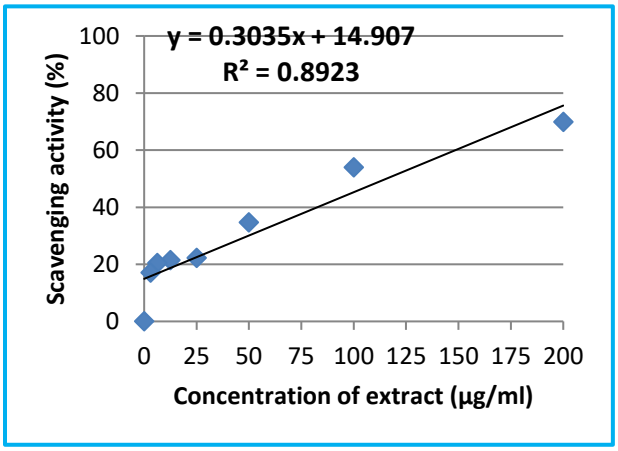


Figure (8): GC-MS chromatogram of methanolic extract of Euphorbia prostrata

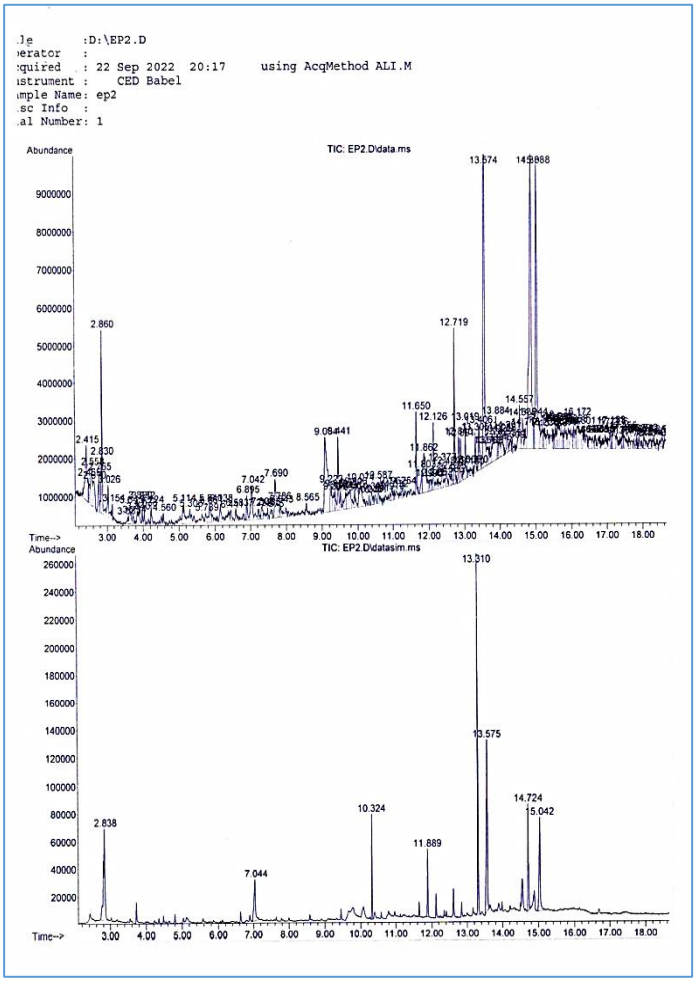
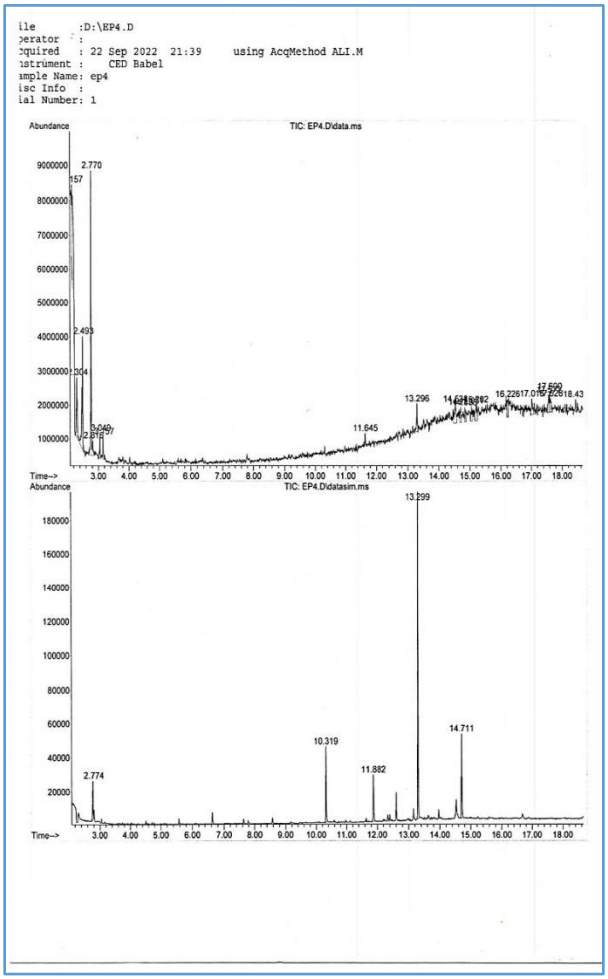


Figure (9): GC-MS chromatogram of methanolic extract of Euphorbia hypericifolia.



4. DISCUSSION:

1: Chemical study .

There is no doubt that all plants may contain similar chemical compounds, but it is not necessary that they be of a degree of kinship, so it has become possible to rely on chemotaxonomy in solving taxonomic problems and at the level of taxonomic ranks.

The results of the current study showed that the study of chemotaxonomy had a good role in separating the two species E.prostrata and E.hypericifolia, as the results of GC-MAS analysis of the methanolic extract recorded different chemical compounds for both species, and other similar compounds were recorded in both species. It was also noted that some of the compounds identified during the results of the analysis are responsible for some pharmacological effects based on some previous studies.. Some compounds have an antibacterial effect , such as 3-(5-Bromo-3-nitro-1H-1,2,4-triazol-1-yl) - 6,8-dioxabicyclo [3,2.1]octan-4-one (Strzelecka and Świątek , 2021) . As an antifungal compound, Propa noic acid, 3-chloro-, ester. Also, as an antioxidant like Camphene, as well as the importance of fatty acids. As several studies indicated the importance of the activity of fatty acids as antibacterial and antifungal . (Yoon et.al, 2018) showed that antimicrobial lipids are single-chain fatty compounds that interact with bacterial cell membranes and exhibit antibacterial activity. The fatty acids consist of single saturated or unsaturated hydrocarbon chain and a carboxylic acid group at one end , the most important of which is the fatty acid Capric acid. It is one of the fatty acids recorded in the results of the current study, as capric acid and its monoglyceride derivative, mono-caprine, contain saturated hydrocarbon chains that are 10 carbons long , and it also has high antibacterial activity, especially against Gram-negative bacteria. (Yoon et.al, 2018) explained that the mechanism of action of fatty acids as antibacterial targets mainly bacterial cell membranes, and in particular works on membrane destabilization and cell lysis, which leads to inhibition of cell growth or death of the bacterial cell . Also ,(Ahmed et.al,2011) indicated that fatty acids have enormous biological importance, and they have a role in declining the risk of heart disease , inflammation and increasing immunity.

2: Antioxidant activity

Antioxidants are substances capable of inhibiting oxidative processes and thus block the chemical reaction that transfers electrons

or hydrogen to an oxidizing agent such as glutathione (Devasagayam et.al, 2004).

Table (1) shows the antioxidant activity of E.prostrata and E.hypericifolia by using different solvents. As a comparative study, during the current study, the antioxidant activity was determined using the DPPH method, as different solvents (Methanol, Ethyl acetate and Hot water) were used to make a plant extract of the leaves of two species E.prostrata and E.hypericifolia. Also, different concentrations were taken (200, 100, 50, 25, 12.5, 6.25, 3.12) (µg/ml) for each solvent.

The results showed that the highest rate of scavenging activity was in the methanolic - aqueous extract, and the lowest rate of activity was in the ethyl acetate and the aqueous extract for both species under study. These results are consistent with the results of the study (Ahmed et.al, 2011) , as they confirmed the high potential of the methanolic extract in terms of free radical scavenging activity. In addition, the study recorded the highest rate of scavenging activity in the methanolic-aqueous extract of E. prostrata , which was (94.02%, 86.14%, 82.08%, 66.95%, 53.65%, 44.59% and 41.16%), while the activity rate was recorded in E. .hypericifolia (79.84, 61.80, 53.02, 46.98, 35.71, 31.29, 24.34) at concentrations µg/ml (200, 100, 50, 25, 12.5, 6.25, 3.12) for both species .

As shown in Table (1), the methanolic-aqueous extract of E. prostata has high antioxidant activity compared to that of ascorbic acid, while E.hypericifolia showed lower effectiveness compared to that of Ascorbic acid except for the highest concentration . The results of the study also confirmed that the methanolic- aqueous extract of E. prostrata has strong antioxidant activity compared to E. hypericifolia , These results were consistent with a study (Ahmed et.al,2011) , and a study (Prabha et.al, 2018) confirmed the high efficacy as an antioxidant of the ethanolic extract of E.prostrata.. These results may be due to the presence of a high percentage of fatty acids, as well as to the presence of some phenolic , flavonoids and terpenes compounds . These results are consistent with the study of (Agbo et.al, 2019) . They indicated that E. prostrat is a medicinal plant and contains a good percentage of phenolic , flavonoids and terpenes compounds, in addition to the presence of some esters and other important compounds.

5. CONCLUSIONS:

In this study, the chemical analysis using GC-MAS technique of the methanolic extract of leaves of E.hypericifolia and E.prostrata revealed many chemical compounds with good biological activity as antibacterial and antifungal , as well as antioxidants. The results also showed that the extract prepared using the aqueous - methanolic solvent has a strong antioxidant activity compared to the other solvents used during the study. The results also showed that the aqueous- methanolic extract of the leaves of E.prostrata has a strong antioxidant activity compared to the type E.hypericifolia .. Thus, the chemical study and the antioxidant activity have good taxonomic importance in separating the two species under study.

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Conflicts of interest and financial disclosures :

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