PHYTOCHEMICAL CONSTITUENTS AND GAS CHROMATOGRAPHY WITH MASS SPECTROSCOPY ANALYSIS OF *EUPHORBIA HETEROPHYLLA*'S AERIAL PARTS

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ABSTRACT

Alkaloids, phenolics, tannins, glycosides, saponins, sterols, and flavonoids among other bioactive secondary metabolites were found in phytochemical analysis. It was found that cardiac glycosides were absent in leaves of the investigated plant whereas anthraquinones were not found in both leaves and stems of the plant. This study identified the plant's total (alkaloids, flavonoids, tannins, saponins and phenolic acids) percentage. The non-polar components of *Euphorbia heterophylla* L. were studied. GC-MS analysis of chloroform extracts and crude hexane, as well as other chromatographic separation procedures, were used to identify and characterise eight known chemicals (using MASS). These compounds: Hotrienol, 1,7-Octadiene-3,6-diol, 2,6-dimethyl, 1,7-Octadiene-3,6-diol, 2,6-dimethyl, Coumarin, Caryophyllene oxide, 2H-1-Benzopyran-2-one, 7-methoxy, Longifolenaldehyde, Hexadecanoic acid and ethyl ester**.**

Keywords: GC-MS, *Euphorbia heterophylla*, phenolics, flavonoids

1. Introduction

Medicinal plants were shown to be renewable resource that include substances with nutritional and pharmaceutical characteristics that enable humans to prevent and treat ailments **(El Sohaimy, *et al.*, 2015)**. Plants are important sources of natural antioxidants that can be used to generate novel medications, and medicinal plants have been designated as a repository for numerous bioactive chemical types with various therapeutic qualities. The therapeutic potential of plants was discovered over a long period of time. The great majority of medicinal plant therapeutic benefits include antioxidants, analgesics, anti-inflammatory, antiviral, anti-tumor and antimalarial characteristics **(El Sohaimy, *et al.*, 2015)**. *Euphorbia heterophylla* belongs to family Euphorbiaceae, also known the spurge family, a huge flowering plant family with about 7,500 species and 300 genera. The majority of spurges are herbs, although some are shrubs or trees, especially in the tropics. Some resemble cactus and are succulent. This family is mostly found in the tropics, with the Indo-Malayan region and tropical America having the most species. Tropical Africa has a great diversity, although not as many or as diverse as these two other tropical regions. However, Euphorbia is the most diverse genus of flowering plants on the planet, with at least 2100 species **(Prenner and rudall, 2007)**. Euphorbia is the third biggest genus of flowering plants in the world, with about 2,000 species distinguished by the presence of milky latex **(Horn, *et al.*, 2012)**. Biologically active phytochemicals with therapeutic effects can be found in some Euphorbia species **(Vasas and Hohmann, 2014)**. Prehistorically records show that Euphorbia species were used in the treatment of scorpion and snake bites, liver diseases, respiratory disorders, asthma and rheumatism in the Chinese and Ayurveda medicine systems **(Kemboi, *et al.*, 2020)**. The medicinal applications of these species have been attributed to the presence of diverse secondary metabolites such as flavonoids and terpenes **(Islam, *et al.*,2016)**. **Alsaffar, *et al.*, (2021)** reported to presence of flavonoids in *E. heterophylla.* **Ajuru, *et al.*, (2017)** indicates positive effect for flavonoids in whole plant of *E. heterophylla* with value 0.28±0.03 using ethanolic and aqueous extracts. According to **Abbasi, *et al.*, (2013)** Phytochemical screening of *E. heterophylla* revealed to flavonoids in abundance in chloroform soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction.The technology of gas chromatography mass spectrometry (GC-MS) has been specifically designed to examine the numerous components present in plant extract, as well as their molecular structure. There are two major advantages of using GC-MS to analyse the bioactive components in medicinal herbs. Firstly, With the capillary column, GC-MS provides a better isolation ability, allowing for a more accurate and precise chemical fingerprint; secondly, The GC-MS with connected mass spectrum database could provide quantitative results on the herbs studied, which will be incredibly important for future research into the interaction between phytoconstituents in phyto-medicinals and their bioactivities. **(Sushma and Arun, 2016)**. GC-MS is particularly effective in identifying pure compounds in trace levels of less than 1 mg. The GC-MS method is particularly useful for detecting pure compounds in tiny levels. thus, there is no information on a detailed GC-MS analysis of this plant's bioactive components. therefore, a full assessment of the ethanol extract of *Euphorbia heterophylla* leaves and stems was performed in order to separate bioactive molecules and therapeutic activities from the powerful components.

2. Materials and Methods

2.1. Plant material:

The plant of collected from Alex-Marsa Matrouh Road, 10Km west of El-Hammam town . The plant sample was identified by Herbarium of Desert Research Center.

2.2. Sample preparation

The leaves and stems (100 g) of *Euphorbia heterophylla* powdered sample were extracted with a least of 70% ethanol and purified depending on standard technique **(Mabry, *et al.* 1970)**, This extract was utilized to determine the chemical component of *Euphorbia heterophylla* on a qualitative and quantitative basis, on another hand GC-MASS analysis requires a little quantity of hexane combined with chloroform (v/v) to extract volatile compounds. The slurry was let to still for one day, stirring occasionally, before filtration. The residue was continuously extracted with a % ethanol or hexane to chloroform (v/v) excess volume. The combined filtrates were evaporated at 60º C for a quarter hour under decreased pressure using a rotavapor apparatus until just a trace of solvent remained.

2.3. Preliminary qualitative phytochemical screening

Using basic phytochemical methods, the ethanolic 70 % crude extracts of *Euphorbia heterophylla* were submitted to preliminary qualitative phytoscreening for the existence of biological active components **(Debiyi and Sofowora, 1978)**.

2.3.1. Flavonoids Test:

a. Shinoda Test: The presence of flavonoid was indicated by the pink color when HCl concentrated and segments of magnesium strip were combined with aqueous extract after a few moments.

b. Glycosides Test: minimal extract quality, mixed with 1 ml water and shaken well, then added a NaOH aqueous solution The presence of glycosides was indicated by a yellow tint.

2.3.2. Cardiac glycosides test:
a. Legal's Test. To treat the extracts, sodium nitroprusside in pyridine as well as sodium hydroxide were utilised. A pink to blood-red colour indicates the presence of cardiac glycosides.

2.3.3. Tannins test:
a.Lead Acetate Test: Only few drops of solution called (10% lead acetate) were combined with 5 ml of extraction. The presence of tannins was detected by the appearance of a yellow or reddish precipitate.

2.3.4. Sterols test: 3 ml of the extracts were completely dried by evaporation. After being dissolved in 2 ml chloroform, the remainder was filtered. The filtrate was treated to (Salkwski Reactions), a 1 ml chloroform extract and concentrated sulphuric acid was

poured down the side of the tube. The appearance of a yellow colored ring that become dark red denoted a positive reaction.

2.3.5. Saponins test:

a. Frothy test: 20 ml of distilled water utilized to dilute the extract by adding then shaking for a quarter hour in a measuring cylinder. A 1 cm layer of foam was used to identify saponins..

2.3.6. Phenols test:

a.Ferric Chloride Test: To the extracts, 3–4 drops of FeCl3 solution were then added. The existence of phenols is revealed by blue-black tint when seen it.

2.3.7. Anthraquinone test:

In a conical flask, for 10 minutes 10 ml of benzene was mixed with 6 g of plant powder sample before filtering. After that, another 10 mL of a 10% ammonia solution was added to the filter and shaken by force for half a minute. The existence of anthraquinones in the ammoniaphase was revealed by the existence of (violet, pink, red) color.

2.4. Total active substances evaluation:

2.4.1. Estimation of total phenolic acids (Li, *et al.*, 2013)

To estimate the quantity of (TPC) total phenolic acids in the extract, the Folin Ciocalteu reagent was utilized. As a standard, gallic acid was utilized, and total phenolics were represented as g/mg gallic acid equal to (GAE). In methanol, gallic acid amounts of 2, 4, 6, 8, and 10 g/ml were made. A plant extract concentration of 1 mg/ml was likewise produced in methanol, The test consisted of mixing 0.5 ml of each sample with 2.5 ml of a 10 fold dilute Folin Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. After closing the tubes with parafilm and allowing them to sit at room temperature for 30 minutes, the absorbance at 760nm was measured spectrophotometrically. All tests were carried out in triplicate. Reduced substances, such as polyphenols, are responsive to the Folin Ciocalteu reagent. As a result of the reaction, they become blue. This blue color was spectrophotometrically measured. For the estimate of unknown phenol content, a line of regression from Gallic acid (GA) was utilized. From the Gallic acid standard curve, The regression line was shown to be

$$y = 0.03R2 + 0.0913x = 0.9976$$

The absorbance is denoted by (y), while the g GAE/mg of the extract is denoted by (x).

Hence, the goodness of fit for the chosen standard curve was determined to be excellent. By putting the test sample's absorbance (y= absorbance) on the regression line of the previously mentioned (GA).

2.4.2. Total saponins estimation: (Okwu and Ukanwa, 2007).

In (200 ml) of 20% ethanol, (20 g) of plant powder was dispersed. At around 55°C, The hot water bath used to heating the suspension for 240 minutes while being shacked constantly. After filtering the mixture, re-extraction of the residue was performed with (200 ml) of 20% ethanol. Using a 90°C water bath, The full content of the mixture was decreased to 40 ml. The concentration was combined with 20 ml diethyl ether in a 250 ml separating funnel and vigorously agitated. The ether layer was discarded, whereas the aqueous layer was maintained. The purification procedure was repeated with the addition of 60 ml of n-butanol. The mixed n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. A water bath was used to heat the remaining solution. To obtain the desired weight, the samples were dried in an oven after evaporation. As a proportion of total saponins, saponin content was calculated.

2.4.3. Total tannins estimation:

Gravimetric procedure: (Copper acetate method) **(Ali, 1991)**. This method entails quantitatively precipitating tannin in a copper acetate solution, burning copper tannate to CuO, and estimating the residual CuO. 2g of plant powder were extracted in two different volumes of 100ml acetone-water (1:1) for roughly a 60 minutes each and then filtered. In both cases, The mixed extract was poured into a ¼ liter volumetric flask and filled with distilled water to the required volume. In a 500ml beaker, the extraction was heated to boiling then adding 30ml of a 15% aqueous copper acetate solution while shaking. The ashless filter paper was used to separate the copper tannate product, which was subsequently burned in a crucible(The crucibles were previously burned at the same temperature and at an uniform weight). The weight was returned after the residue was treated with a few drops of nitric acid. The following correlation was used to evaluate the weight of Copper oxide and the amount of tannin: 1 g of Copper oxide is equivalent to 1.305 g of tannin.

2.4.4. Total alkaloids Estimation:

(Gravimetric method) **(Woo, *et al.*, 1977).** 90% ethanol was utilized for extract Approximately 10 g of plant powders until exhaustion (as determined by reagent of Mayer). At a temperature of 40 °C, the alcoholic plant extract was concentrated under reduced pressure until it became dry, then acidified with HCl (3%) and filtered The acid alkaloid component of the filtrate was extracted using chloroform. Chloroform was used to remove the alkaloid basic component until it was exhausted, and ammonia was used to modify the acidic aqueous layer to an alkaline medium (as determined by reagents of Mayer and Dragendorrf). Anhydrous sodium sulphate was used to filter the chloroform extract. It was then evaporated at decreasing pressure until entirely dry, and the percent was estimated by weighing it (w/w).

2.4.5. Total Flavonoid Content (TFC) **(Malla*, et al.*, 2013)**.

Colorimetric analysis was used to evaluate the quantity of Total contents of Flavonoids in extracts using an aluminium chloride assay. A 2 ml distilled water was combined with 0.5ml aliquot of adequately diluted sample solution , then 0.15 ml of a Sodium nitrite 5% solution was added. During 6 min, 0.15 ml of an Aluminum chloride 10% solution was added and allowed to stand for another 6 min before being combined with 2 ml of a Sodium hydroxide 4% solution. Water was immediately poured to raise the definitive volume to 5 ml, following that, the mixture was well combined one again, then set aside for a quarter hour. In comparison to a produced water blank, the absorption of the mixture was evaluated at 510 nanometers. For the estimation of total flavonoid, rutin was utilised as a control component. Rutin's calibration curve has been used to calculate content of total flavonoid in mg rutin/g dry mass (mg rutin/g DW). Three replications of each sample were performed.

$$y = 0.0029x + 0.0034 R2 = 0.9935$$

The absorbance of the extract was (y), while the rutin/mg was (x). so, the fit goodness for a certain standard curve was found to be satisfactory. By inserting the test sample absorbance (y = absorbance) in the line of regression of the rutin previously described.

2.5. Qualitative and quantitative determination compounds of *Euphorbia hetrophylla* plant using GC-MASS:

GC-MASS was carried out according to Gas chromatography–mass spectro-metry (GC-MS) analysis: By comparing mass spectra and retention times with those of the NIST 11 and WILEY 09 mass spectral databases, the chemical composition of plant extract was separated, purified, and characterized. **(Swidan *et al.*, 2020)**.

3. Results and Discussion:

Chemical analysis is used to determine the quantitative and qualitative composition of multiple biochemical ingredients, which helps in the detection of active structures that cause the plant to respond biologically. The biological active secondary compounds such as saponins, flavonoids, tannins, phenolics, glycosides, sterols, and alkaloids was discovered in this study through preliminary phytochemical screening of *Euphorbia heterophylla* leaves and stems. Anthraquinones were absent from the studied plant but were Cardiac glycosides not found in the leaves of the plant, morever they were found in the stems, as shown in the table (1).

On the respect **Kayani *et al.*, (2007)** revealed that Secondary metabolites are created by secondary reactions coming from primary metabolites such as lipids, carbohydrates, and amino acids . These phytochemical elements are biologically important and have hypolipidemic, anti-tumor, or stimulating effects that can help to minimise the risk of cancer and heart disease. Furthermore **Ajuru, *et al.*, (2017)** indicates positive effect for All active compounds (Alkaloid ,Anthraquinone, Coumarin, Flavonoid, Phenols, Quinone, Saponin, Tannin, Sugar, Glycoside) were present in ethanol extract from *Euphorbia heterophylla*, while aqueous extract of *Euphorbia heterophylla* revealed for

Alkaloid, Flavonoid, Phenols, , Saponin, Tannin, Sugar, Glycoside except quinone , Anthraquinone and Coumarin.

According to the result shown in table (2) total active materials in *Euphorbia heterophylla* leaves and stems like flavonoids reached to (276±0.69, 250.6±0.78) mg/gm rutin and total phenolics reached to (363.6±0.97, 301±0.57) mg/gm Gallic acid. In addition to its antioxidant and free radical terminator properties of *Euphorbia heterophylla* it was reported **(Bendini, *et al.*, 2006)** that a number of bioactivities (flavonoids and other plant phenolics) were observed in *Passiflora spp.* Extract related to antioxidant and antimicrobial activities. As a result, calculating their total amount in the plant's leaves and stems is beneficial. On another hand each of total saponins, tannins and alkaloids were (1.7±.10, 1.43±0.13), (1.8±0.10, 1.3±0.17) and (1.66±0.15, 1.13±0.13) respectively.

GC-MS is one of the most effective methods for determining esters, acids, alcohols, branched chain hydrocarbons, volatile matter components, long chain and other chemicals. The phytochemical substances were confirmed using molecular formula, retention time, and peak area. The active principles are listed, along with their molecular weight (MW), molecular formula (MF), retention time (RT) and peak area in percent. The presence of 8 chemicals (phytochemical ingredients) in an ethanolic extract of *Euphorbia heterophylla* leaves and stems revealed the medicinal quality of the plant, according to GC/MS analysis (Table 3).

GC-MS has been used to profile metabolites in plant species for the past few years. However, gas chromatography mass spectrometry is only available in a few plant research labs. The identified compounds occupy many biological properties such as anti-inflammatory **(Aparna, *et al.*, 2012)**, Antioxidant, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor **(Kumar *et al.*, 2010**), potentmo- squitolarvicide **(Rahuman, *et al.*, 2000)**.

Gas chromatography–mass spectro-metry investigation of phytochemical substances can be used to identify a plant's therapeutic value. As a result, this type of Gas chromatography–mass spectro-metry investigation is the starting point toward understanding the constitution of therapeutic properties in this natural herb, this kind of investigation will be useful for more detailed study.

Further research is necessary to confirm the pharmacological importance and phytochemistry of *Euphorbia heterophylla.* On another hand biological effect of separation compound : Hotrienol, 1,7-Octadiene-3,6-diol, 2,6-dimethyl, 1,7-Octadiene-3,6-diol, 2,6-dimethyl, Coumarin (figure 2) and Caryophyllene oxide, 2H-1-Benzopyran-2-one, 7-methoxy, Longifolenaldehyde, Hexadecanoic acid, ethyl ester (figure 3) reflect the important value of *Euphorbia heterophylla.*

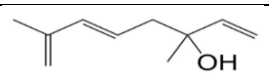
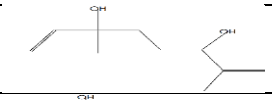
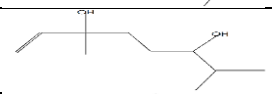
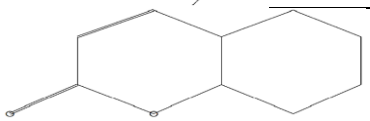
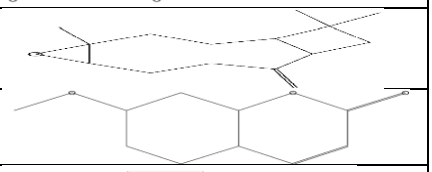
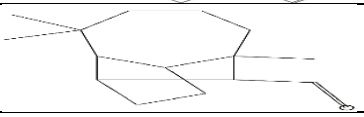
Table (1): Phytochemical screening of *Euphorbia heterophylla*

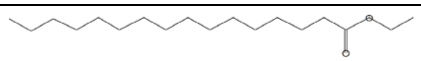
Bioactive constituents	Leaf	Stem
Flavonoids	+ve	+ve
Phenolics	+ve	+ve
Saponins	+ve	+ve
Glycosides	-ve	+ve
Cardiac glycosides	+ve	+ve
Tannins	+ve	+ve
Alkaloids	+ve	+ve
Sterols	+ve	+ve
Anthraquinones	-ve	-ve

+ve: present –ve: absence

Table (2): Total active compounds of *Euphorbia heterophylla*

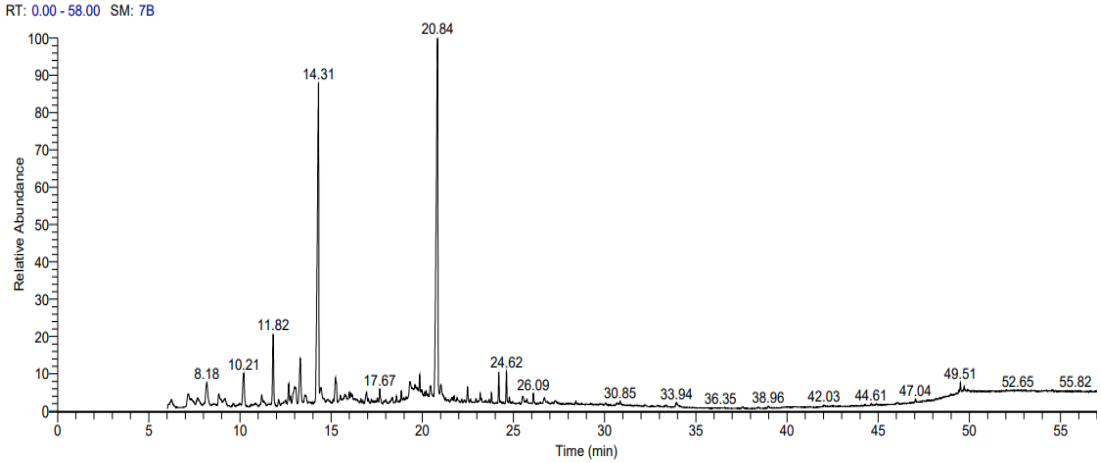
Item	Leaf	Stem
Total flavonoids (mg/gm rutin)	276±0.69	250.6±0.78
Total phenolic acids (mg/gm Gallic acid)	363.6±0.97	301±0.57
Total Saponins (%)	1.7±.10	1.43±0.13
Total Tannins (%)	1.8±0.10	1.3±0.17
Total Alkaloids (%)	1.66±0.15	1.13±0.13

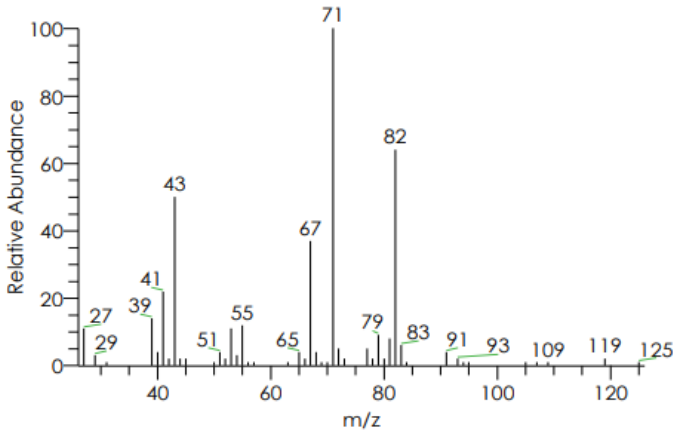
No.	R.T.	Peak Area(%)	Name of compounds	Molecular formula	Molecular weight	Chemical structure
1	**8.18**	**2.20**	**Hotrienol**	**$C_{10}H_{16}O$**	**152**	
2	**10.21**	**3.19**	**1,7-Octadiene-3,6-diol, 2,6-dimethyl**	**$C_{10}H_{18}O_2$**	**170**	
3	**11.82**	**4.89**	**1,7-Octadiene-3,6-diol, 2,6-dimethyl**	**$C_{10}H_{18}O_2$**	**170**	
4	**14.31**	**27.87**	**Coumarin**	**$C_9H_6O_2$**	**146**	
5	**17.67**	**0.89**	**Caryophyllene oxide**	**$C_{15}H_{24}O$**	**220**	
6	**20.84**	**32.33**	**2H-1-Benzopyran-2-one, 7-methoxy**	**$C_{10}H_8O_3$**	**176**	
7	**24.62**	**1.76**	**Longifolenaldehyde**	**$C_{15}H_{24}O$**	**220**	

8	**26.09**	**0.62**	**Hexadecanoic acid, ethyl ester**	**$C_{18}H_{36}O_2$**	**284**	

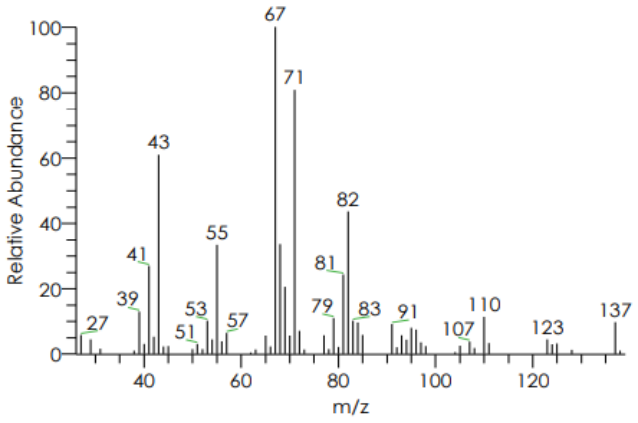
Data are presented as mean ± SE for 3 replicates.

Table (3): GC- MASS analysis of *Euphorbia heterophylla*

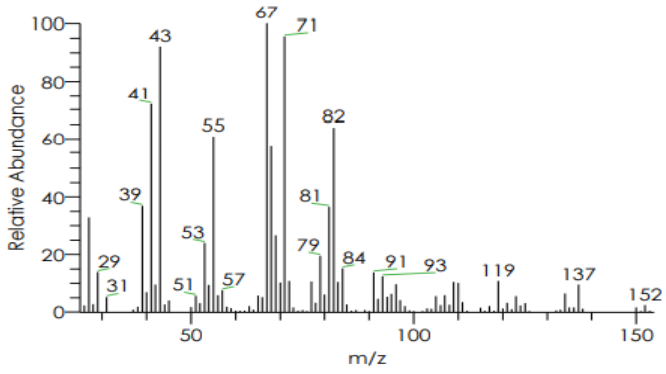




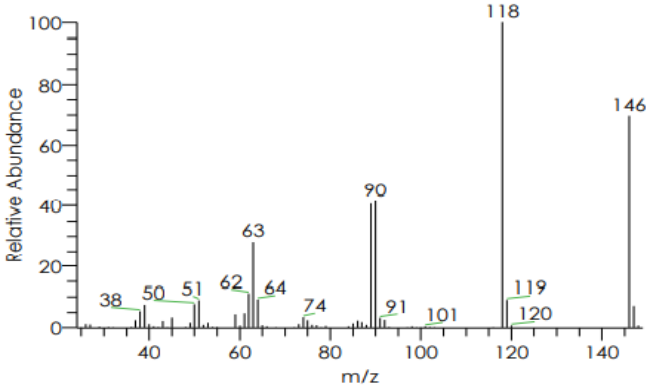
Comp.(1): Hotrienol



Comp.(2): 1,7-Octadiene-3,6-diol, 2,6-dimethyl



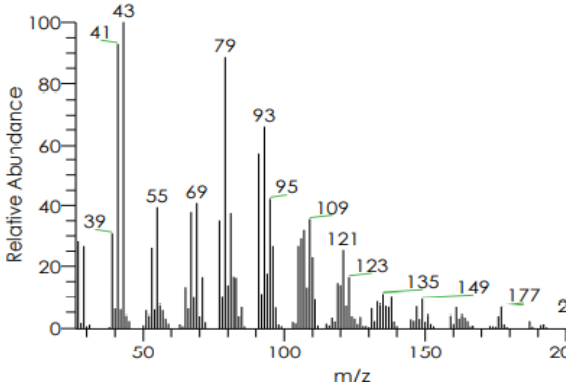
Comp.(3): 1,7-Octadiene-3,6-diol, 2,6-dimethyl



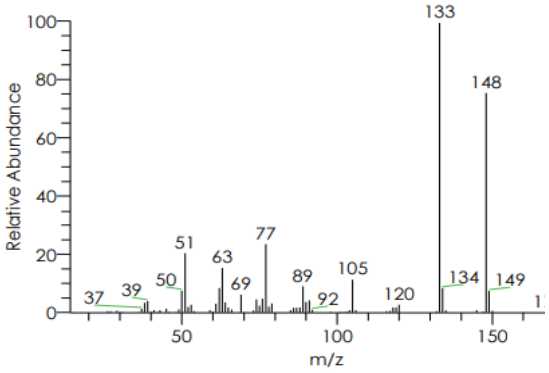
Comp.(4): Coumarin

Fig (1): GC-MS spectral chromatogram of *Euphorbia heterophylla*

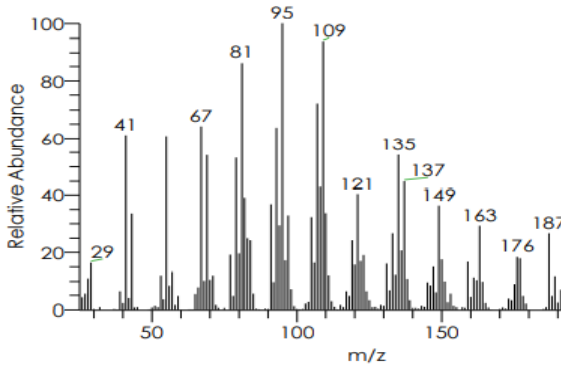
Figure (2) : MASS spectrum for main compounds separation from *Euphorbia heterophylla*



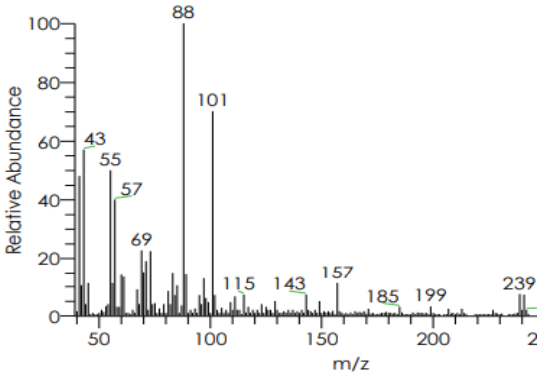
Comp.(5): Caryophyllene oxide



Comp.(6): 2H-1-Benzopyran-2-one, 7-methoxy



Comp.(7): Longifolenaldehyde



Comp.(8): Hexadecanoic acid, ethyl ester

Figure (3) : MASS spectrum for main compounds separation from *Euphorbia heterophylla*

4. Conclusion

The presence of medicinally valuable bioactive components such as tannins, phenolics, saponins, flavonoids, Cardiac glycosides, sterols, and alkaloids were revealed by Gas chromatography–mass spectro-metry investigation of the ethanolic extract of *Euphorbia heterophylla*. Given the medical usefulness of the same components in a various plant extracts, it's no wonder that these components in *Euphorbia* leaves and stems could be just as effective. Work is currently underway to determine its biological activity and improve its pharmacological profile in conventional medicine.

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المكونات الكيميائية النباتية وتحليل كروماتوجرافيا الغاز والمصاحب لها التحليل الطيفي للكتلة للأجزاء الهوائية لنبات لبن الحمارة

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المسح المبدئي لنبات لبن الحمارة أظهر وجود العديد من المواد الأيضية الأولية والثانوية ذات التأثير الطبي حيث تم التعرف على وجود كلا من الفلافونيدات و الفينولات و الصابونينات كذلك الجليكوسيد و التانينات و أخيرا القلويدات في حين لم يتم التعرف على وجود الجليكوسيدات القلبية في اوراق النبات والأنثركينولات في كلا من السيقان والاوراق فى حين أظهر التحليل الكمي للنبات موضوع البحث وجود المواد الأيضية الثانوية (الفلافونيدات – الفينولات – الصابونينات – التانينات – القلويدات) بصوره تحثنا على استخدامه كمصدر للمواد المضادة للأكسدة ذات الاثر الطبي المميز. بعمل مستخلص غير قطبي لنبات لبن الحمارة باستخدام مذيبات غير قطبيه (هكسان وكلوروفورم) وحقن المستخلص المتطاير الناتج على جهاز الفصل الكروماتوجرافى الغازي اتضح وجود 8 مركبات اساسية وهي :

Hotrienol, 1,7-Octadiene-3,6-diol, 2,6-dimethyl, 1,7-Octadiene-3,6-diol, 2,6-dimethyl, Coumarin, Caryophyllene oxide, 2H-1-Benzopyran-2-one, 7-methoxy, Longifolenaldehyde, Hexadecanoic acid, ethyl ester

الكلمات المفتاحية: التحليل الطيفي الكتلي للغاز, نبات لبن الحمارة ,الفينولات , الفلافونيدات