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Determination of Active Phytochemical Compounds of *Alhagi Maurorum* using Gas Chromatography-Mass Spectroscopy (GC-MS)

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Abstract
Alhagi maurorum (camel thorn) is a grayish, evergreen, deeply rooting plant that has spiny needle -like branches. In our study, the phytochemical contents of the root ethanoloic extract of *A. maurorum* were determined by using gas chromatography-mass spectroscopy (GC-MS). Thirty two chemical constituents were identified. We revealed the existence of oxalic acid, anti-2 acetoxyacetaldoxime, sulfone, butyl isopropyl, 2,3-pentanedione, 2-butanone, n,n,o triacetylhydroxylamine. di(1,2,5-oxadiazole)[3,4-b;3,4-e]pyrazine, isobutane,3,4-hexanedione,3-hexanone, pentane, 3-pentanone, 3-butene, 2-thiopheneacetic acid, 2-pyrazoline, 4-hepten-3-onemethylphosphonic acid, butane, propanoic acid, methane, azetidine, heptane, butanoic acid, 4-heptanone, 3,5-dimethyl-4-octane, 3-methoxy-1-pentene, oxirane, 2-undecen-4-ol, 1-heptane, 3- methoxy, acrolein, dimethylacetal, hydrogen azide, ethylenimine, acetic acid, and decane. The results showed that the roots of *A. marrourum* have active compounds that are known to have anti-inflammatory, anti-cancer, antibacterial, anti-ulcerogenic, anti-fungal, anticancer, and anti-malarial activities.

Keywords: A. maurorum, phytochemical compounds, GC-Ms.

تحديد المركبات الكيميائية النباتية النشطة لنبات العاقول بأستخدام جهاز التحليل الطيفي للكتلة الغازية GC–MS

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الخلاصة
نبات العاقول هو نبات رمادي،دائم الخضرة,عميق الجذور و له فروع شوكية تشبه الابر. في دراستنا تم تحديد المحتوى الكيمونباتي لنبات العاقول بواسطة تقنية التحليل الطيفي للكتلة الغازية. وتم تحديد اثنين و ثلاثون مكونا كيميائيا من المستخلص الايثانولي لنبات العاقول: حامض الاوكزاليك , مضاد ثنائي اسيتوكسي اسيتالدوكسيم و سلوفون و بوتيل ايزوبروبيل و ٢،٣ – بينتاديون و ٢ –بوتانونثنائي اسيتوكسي اسيتالدوكسيم و سلوفون و بوتيل ايزوبروبيل و ٣،٢ – بينتاديون و ٢ –بوتانون ,N,N,O– triacetylhydroxylamine، ايزوبيوتان ، ٤،٣– هيكسانوديون ، ٣ –هيكسانون ، بينتان، ٣ –بنتانون ، ٣ –بيوتين، حمض ٢ – ثيوفينيكتيك، ٢– pyrazoline وحمض الفوسفات المثيلي، ٤ –هيبتان – ٣ –واحد ، بيوتان، حمض البروبانيك، ميثان، ازيتيدين، هيبتان، حمض البيوتانويك ، ٤– هيبتانون، ٣،٥ – ثنائي مثيل – ٤ –اوكتان، بنتين، اوكسيران ، ٢ –اونديسين – ٤ –اول ، ١ –هيبتان، ٣ –ميثوكسي اكرولين ، ثنائي ميثيل اسيتال، ازيد

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الهيدروجين، امينات الاثيلين، حمض الخليك، ديكان.اظهرت النتائج احتواء جذور نبات العاقول على العديد من المركبات الفعالة التي لها العديد من التأثيرات كمضادة للالتهابات، ومضادة للسرطان وضد البكتيريا، وضد التقرح، وضد الفطريات، ومانعة لتكوين السرطان وضد فعالية الملاريا.

Introduction

The plant *Alhagi maurorum* is the deepest rooted compared with other plants, with roots that reach 7 feet deep into the ground, while the plant is around 4 feet in height. The plant, which is grayish- green in color, has small, internal leaves that have reciprocal arrangements. The plant has small oval leaves and little flowers. The fruits are brawny in color and tend to be found near the seeds, with a little peak. Traditional medicine is considered to be the oldest type of treatment that has been continuously used in treating some diseases in many countries of the world. Studies found that the plant contains many active compounds, such as flavonoids, triterpenes, coumarins, glycosides, sterols, steroids, resins, vitamins, tannins, carbohydrates, alkaloids, unsaturated sterols, and fatty acids.

These compounds are mainly used for their antibacterial, anti-inflammatory, antipyretic, analgesic, antioxidant, gastrointestinal, cardiovascular, diuretic, and many other effects [1]

It was demonstrated that the alcoholic extract of *A. maurorum* can reduce the symptoms of diarrhea with an oral dose between 200 and 400 mg/kg. This action is mediated by the closure of calcium channel [2]. In herbal medicine, *A. maurorum* is used to treat some disorders, such as bilharzia, rheumatism, liver problems, and urinary and digestive diseases [3]. The oil of the floral parts was reported to be used in the treatment of several complicated conditions, such as piles, migraine, warts, and rheumatism. Water extracts of the plant roots were given for ureter enlargement to eliminate kidney stones [4, 5].

The plant is mainly found in Arab countries, being native to Arabian deserts, with popular names such as Al-Aqool, Al-Shook, or camel-throne [6] [7]. *A. maurorum* is one of the wild plants that are widespread in Iraq. Due to the scarcity of references about its medicinal uses in Iraq, this study aimed to investigate the active compounds in the roots of *A. maurorum* and its pharmaceutical importance, paving the road for later studies on its effects on cell division.

Materials and Methods

Plant collections

This work was carried out in the laboratories of the Biology Department, College of Science**,** University of Baghdad, Baghdad, Iraq, during the period 1/9/2020 - 15/9/2020 (fig 1). The roots of *A. maurorum* plant were obtained from some of their natural habitats in Baghdad. The roots were separated from the soil and washed well with tap water to remove dust. The roots were then cut into small pieces and placed in an oven for drying at 40°C for 72 hrs. They were then mixed in a blender until a powder was formed [8]**.** The roots powder was stored in the refrigerator with a piece of charcoal to prevent humidity.

Figure 1- Alhagi maurorum plant in the garedens of Univercity of Baghdad

Preparation of Plant Extracts

The plant roots were prepared by using Soxhlet apparatus. 20 g of the weighed plant root powder was placed in a thimble with 500 ml hydro-ethanol (ethanol 50%) for 8 hrs. The solution was filtered through muslin cloth. The filtrate was evaporated under reduced pressure by using a rotary evaporator and vacuum dried. The plant extract was transferred to a glass sealed cans and placed in the refrigerator before the extraction process [9].

Gas chromatography-mass spectroscopy analysis

The chemical constituents of *A. maurorum* extract were determined via using a GC-MS technique. The parameters of this experiment were determined as follows: TR 5-MS capillary standard non-polar column, length: 30 m, width: 0.25 mm, film: 0.25 mm. The rating flow of the mobile phase (carrier gas: He) was set at 1.0 ml/min. The temperature of the GC was increased from 400 ºC to 2500 ºC at 50 min The volume of the sample injected in the GC pipe was 1 ml. The sample was left to melt with chloroform to achieve more ideal results. At last, one comparison between the ending consequences of analysis and the parameters of Wiley spectral parameters was made to find the real compounds and their amounts in the tested specimens. The time of this reaction was 36 min [10].

Results and Discussion

The results of gas chromatography analysis of *A. maurorum* root extract showed the presence of fifteen peaks of active chemical compounds. The compounds, as well as their formulae and molecular weights, are as shown in table (1). The GC-MS chromatogram analysis of the alcoholic extract of *A. maurorum* showed the presence of 15 major peaks (Figure 2). The components belonging to these peaks are listed in Table 1. The first set of peak was determined to include oxalic acid, anti-2-acetoxyacetaldoxime, sulfone butyl isopropyl, 2, 3-pentanedione, and 2-butanone, as shown in figures (3-32)

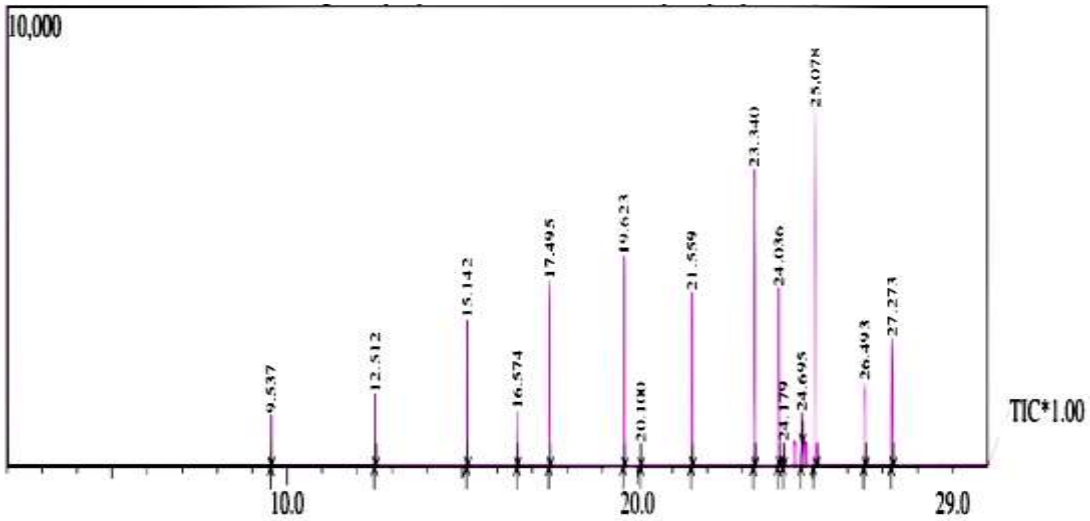

Figure 2- Gas Chromotography-MS profile of *A. maurorum* roots showed the presence of 15 major peaks.

Table 1-Compounds present in the roots extract of *A. maurorum using* GC-MS analysis.

Peak number	Compound name	RT (min)	Chemical formula	Molecular weight	Structure
Peak 1	Oxalic acid	9.533	C9H16O4	188	Fig. 3
	anti-2-Acetoxyacetaldoxime		C4H7NO3	117	Fig.4
	Sulfone, butyl isopropyl		C7H16O2S	164	Fig. 5
	2,3-Pentanedione		C5H8O2	100	Fig. 6
	2-Butanone		C6H10O3	130	Fig. 7
Peak 2	N,N,O Triacetylhydroxylamine	12.508	C6H9NO4	159	Fig. 8
	Di(1,2,5-oxadiazolo)[3,4-b;3,4-E]pyrazine		C8H6N6O4	250	Fig.9
	Isobutane		C4H10	58	Fig. 10
Peak 3	3,4-Hexanedione	15.142	C9H16O2	156	Fig. 11
	3-Hexanone		C6H12O	100	Fig. 12
	Pentane, 1,3- Epoxy-4-methyl-		C6H12O	100	Fig. 13
	3-Pentanone, 2-methyl-		C6H12O	100	Fig.14
Peak 4	3-Butene-1,2-diol, 1-(2-furanyl)-	16.575	C8H10O3	154	Fig. 15
	2-Thiopheneacetic acid		C13H20O2S	240	Fig. 16
	2-Pyrazoline		C8H16N2	140	Fig. 17
	4-Hepten-3-one		C8H14O	126	Fig. 18
	Methylphosphonic acid, dinonyl ester		C19H41O3P	348	Fig. 19
Peak 5	Butane, 2,2-dimethyl-	17.492	C6H14	86	Fig. 20
	Propanoic acid,		C7H12O2	128	Fig. 21
Peak 6	3-Hexanone	19.625	C6H12O	100	Fig. 12
	Isobutane		C4H10	58	Fig. 10
Peak 7	Propane, 2-methyl-1-nitro-	20.100	C4H9NO2	103	Fig. 10
	Methane, isocyanato-		C2H3NO	57	Fig. 22
	Azetidine		C3H7N	57	Fig. 23
Peak 8	3,4-Hexanedione	21.558	C9H16O2	156	Fig. 11
Peak 9	Heptane	23.342	C10H22	142	Fig. 24
	Butanoic acid		C7H12O2	128	Fig. 25
	4-Heptanone		C8H16O	128	Fig. 26
	3,5-Dimethyl-4-octanone		C10H20O	156	Fig. 27
Peak 10	3-Methoxy-1-pentene	24.033	C6H12O	100	Fig. 28
	Oxirane		C4H7BrO	150	Fig. 29
	2-Undecen-4-ol		C11H22O	170	Fig. 30
	1-Heptane, 3- methoxy		C8H16O	128	Fig. 31
	Acrolein,dimethyl acetal		C5H10O2	102	Fig. 32
Peak 11	Hydrogen azide	24.175	HN3	43	Fig. 33
	Ethylenimine		C2H5N	43	Fig. 34
	Acetic acid, anhydride		C4H6O3	102	Fig. 35
Peak 12	anti-2-Acetoxyacetaldoxime	24.692	C4H7NO3	117	Fig.4
Peak 13	Decane, 6-ethyl-2-methyl-	25.075	C13H28	184	Fig. 36
Peak 14	Oxalic acid	26.492	C9H16O4	188	Fig. 3
Peak 15	Pentane, 1,3- Epoxy-4-methyl-	27.275	C6H12O	100	Fig. 13

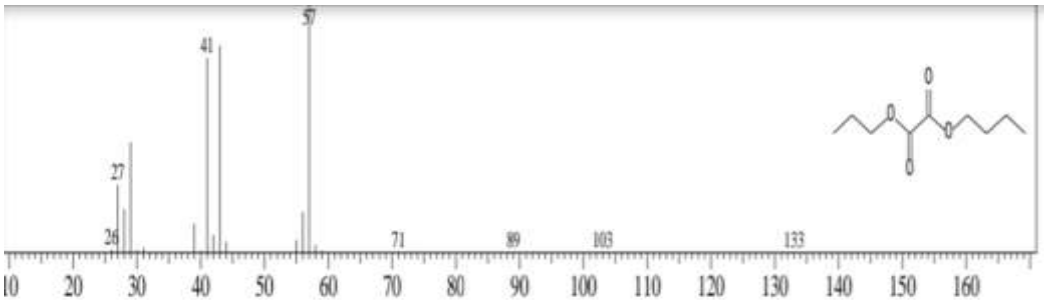

Figure 3-Structure of oxalic acid in roots of *A. maurorum* using GC-MS.

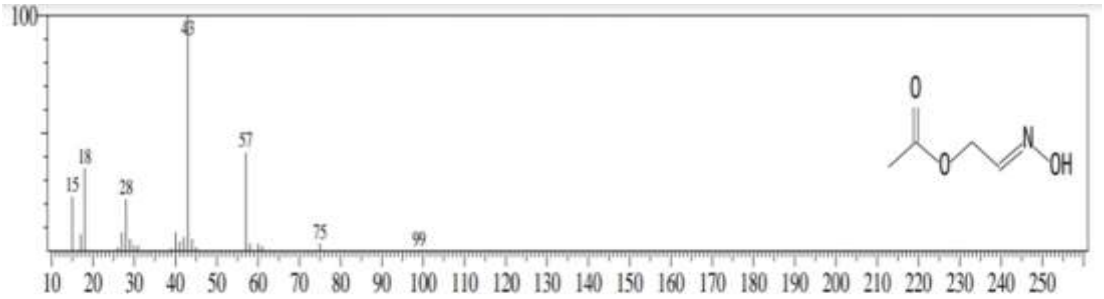

Figure 4-Structure of anti-2a-cetoxyacetaldoxime in roots of *A. maurorum* using GC-MS

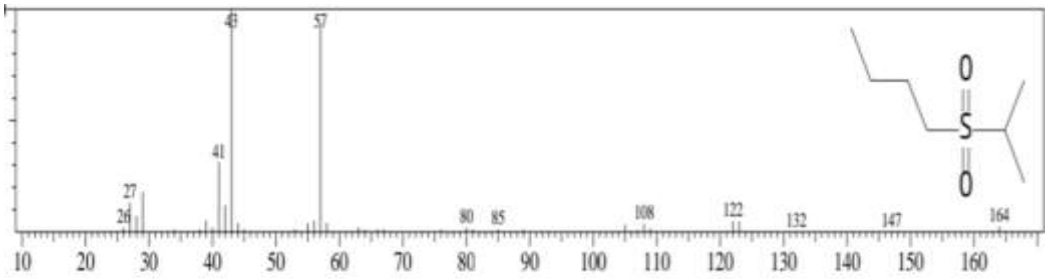

Figure 5- Structure of sulphone in roots of *A. maurorum* using GC-MS

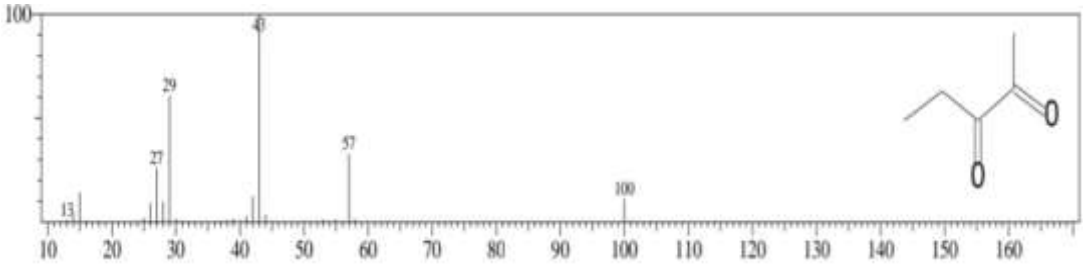

Figure 6-Structure of 2,3- pentadione in roots of *A. maurorum* using GC-MS.

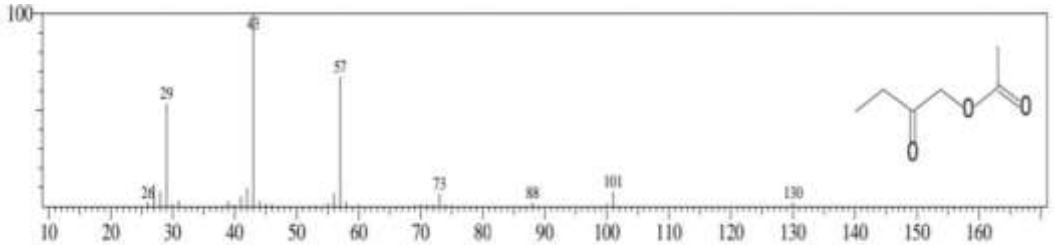

Figure 7- Structure of 2- Butanone in roots of *A. maurorum* using GC-MS.

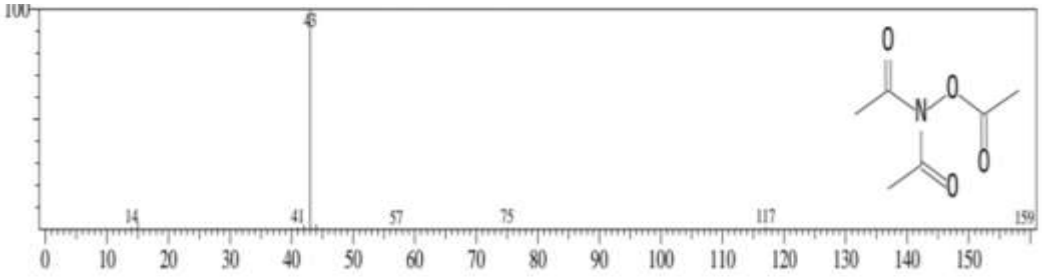

Figure 8-Structure of N,N,O triacetylhydroxylamine in roots of *A. maurorum* using GC-MS.

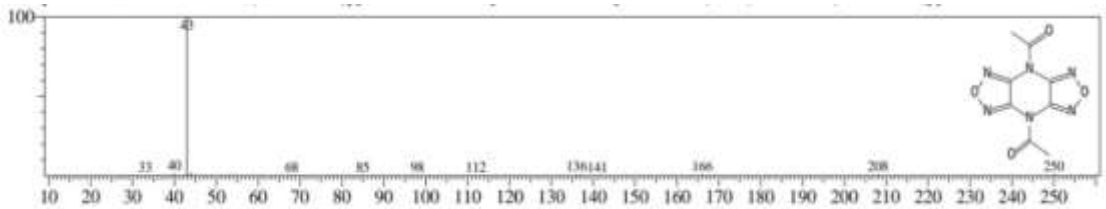

Fig. 9: Structure of di(1,2,5-oxadiazolo)[3,4-b;3,4-E]pyrazine in roots of *A. maurorum* using GC-MS.

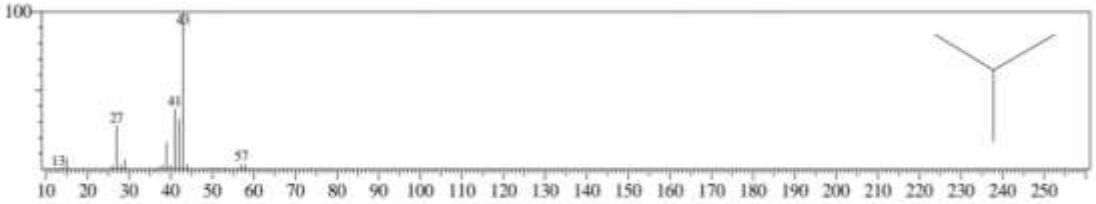

Fig. 10: Structure of isobutene in roots of *A. maurorum* using GC-MS.

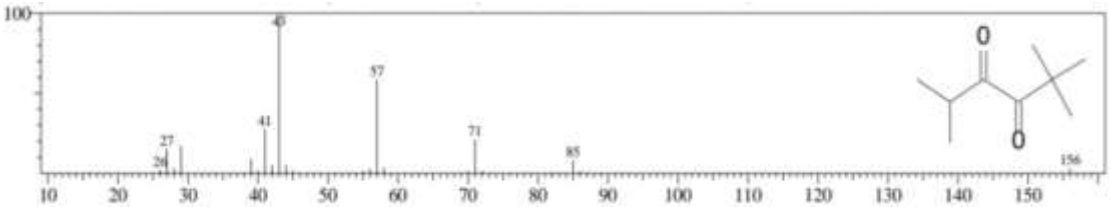

Fig. 11: Structure of 3,4- hexanedione in roots of *A. maurorum* using GC-MS.

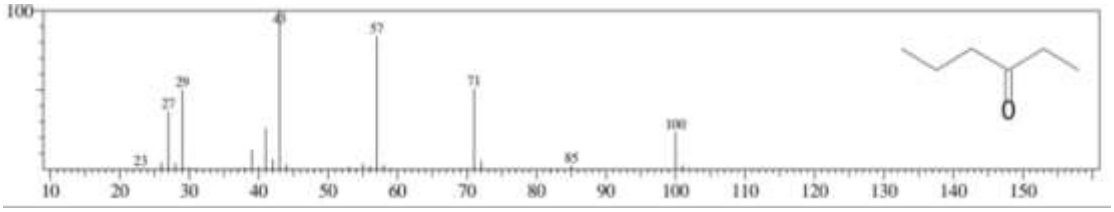

Fig. 12: Structure of 3- hexanone in roots of *A. maurorum* using GC-MS.

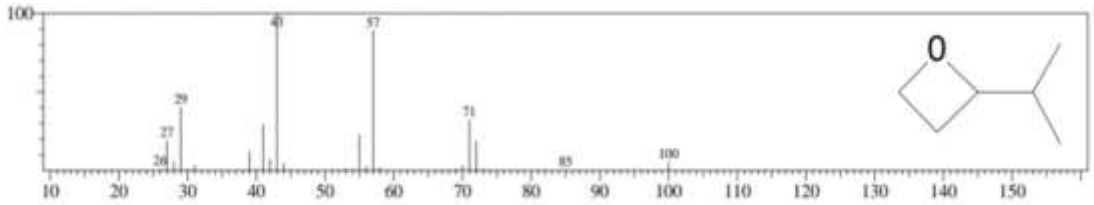

Fig. 13: Structure of pentane in roots of *A. maurorum* using GC-MS.

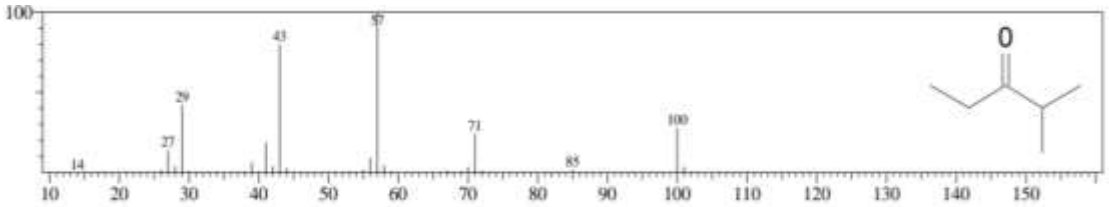

Fig. 14: Structure of 3- pentanone in roots of *A. maurorum* using GC-MS.

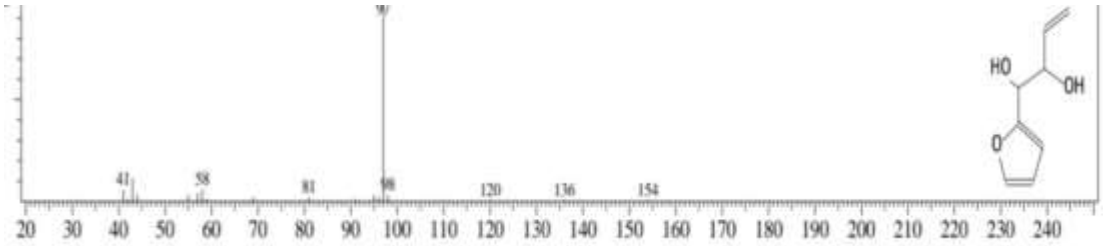

Fig. 15: Structure of 3-Butene-1,2-diol, 1-(2-furanyl)- in roots of *A. maurorum* using GC-MS.

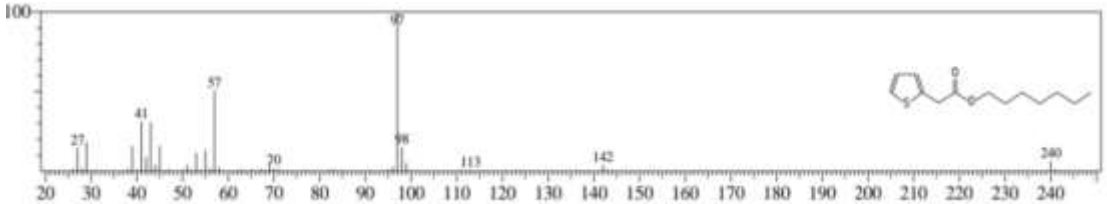

Fig. 16: Structure of 2- thiophenacetic acid in roots of *A. maurorum* using GC-MS.

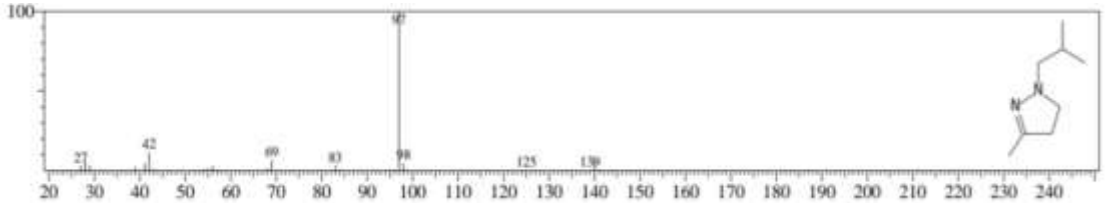

Fig. 17: Structure of 2- pyrazoline in roots of *A. maurorum* using GC-MS.

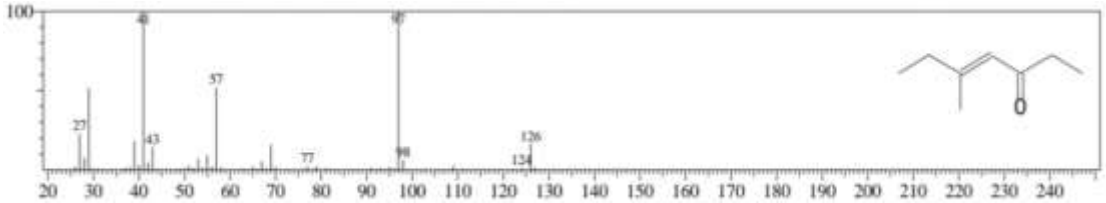

Fig. 18: Structure of 4- heptane -3- one in roots of *A. maurorum* using GC-MS.

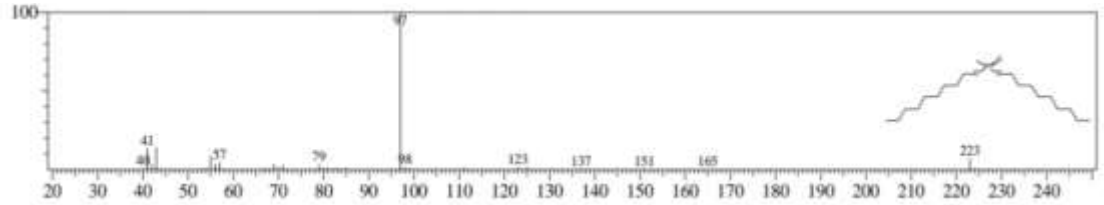

Fig. 19: Structure of methyl phosphonic acid in roots of *A. maurorum* using GC-MS.

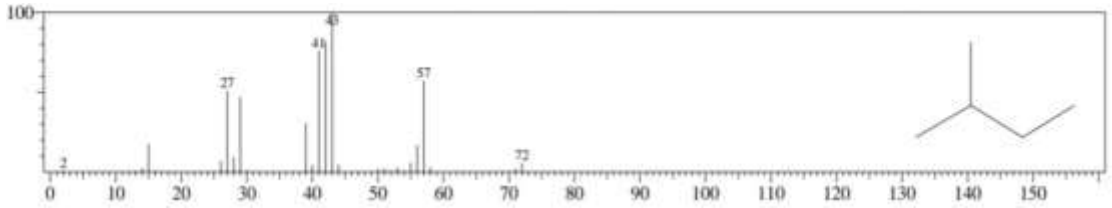

Fig. 20: Structure of butane, 2,2- dimethyl in roots of *A. maurorum* using GC-MS.

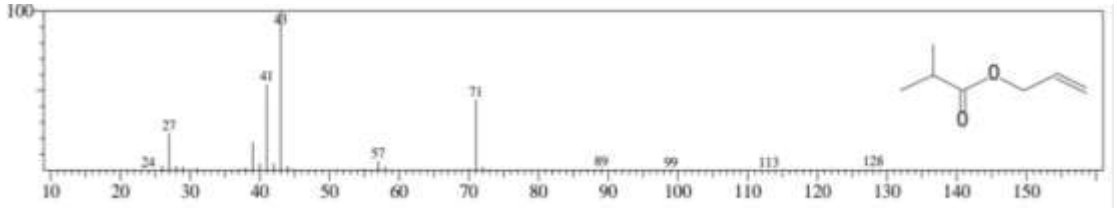

Fig. 21: Structure of propanoic acid in roots of *A. maurorum* using GC-MS.

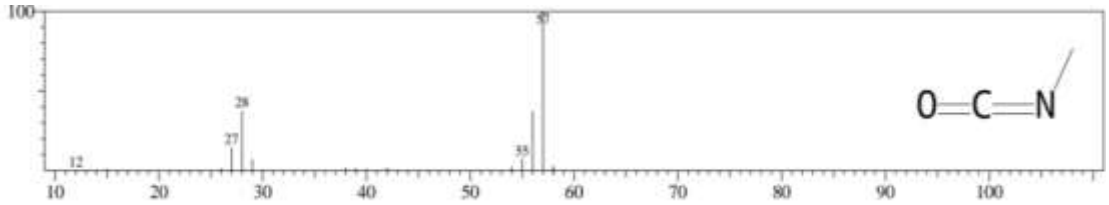

Fig. 22: Structure of methane in roots of *A. maurorum* using GC-MS.

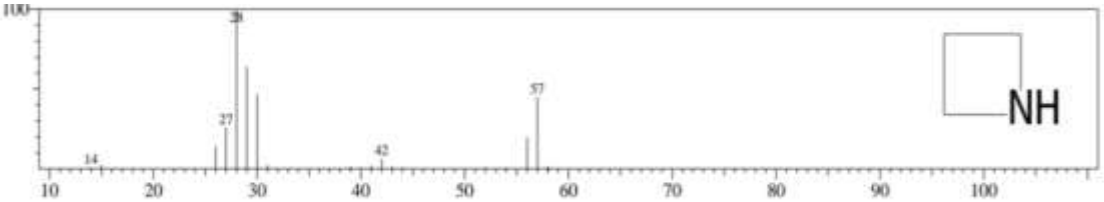

Fig. 23: Structure of Azitidine in roots of *A. maurorum* using GC-MS.

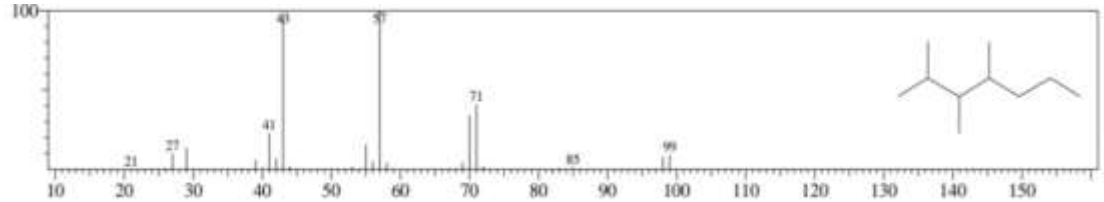

Fig. 24: Structure of heptane in roots of *A. maurorum* using GC-MS.

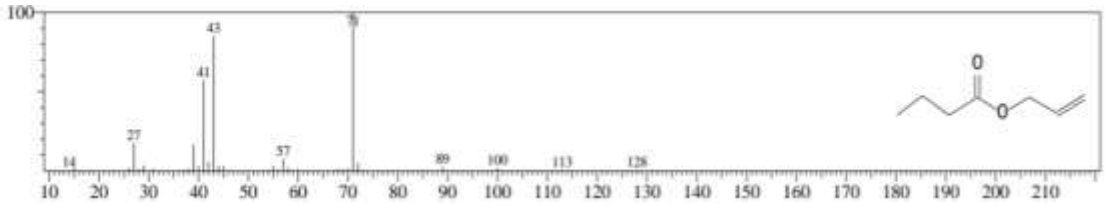

Fig. 25: Structure of butanoic acid in roots of *A. maurorum* using GC-MS.

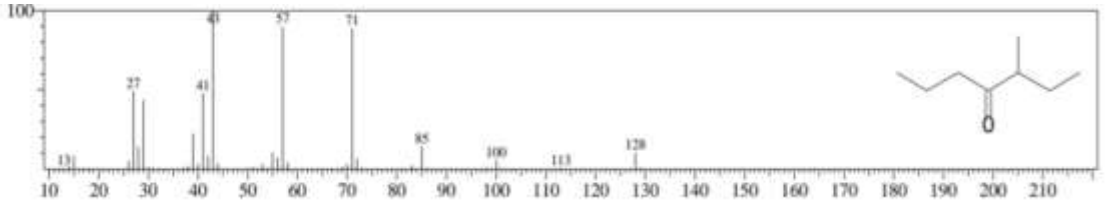

Fig. 26: Structure of 4- heptanone in roots of *A. maurorum* using GC-MS.

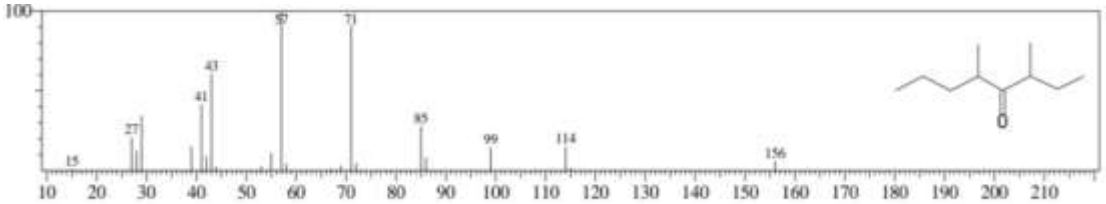

Fig. 27: Structure of 3,5- dimethyl -4- octane in roots of *A. maurorum* using GC-MS.

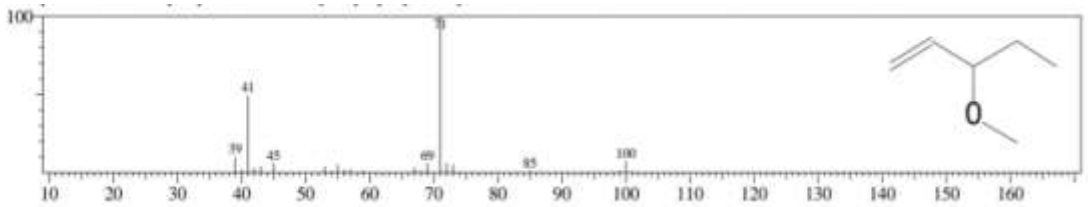

Fig. 28: Structure of 3- methoxy -1- pentane in roots of *A. maurorum* using GC-MS.

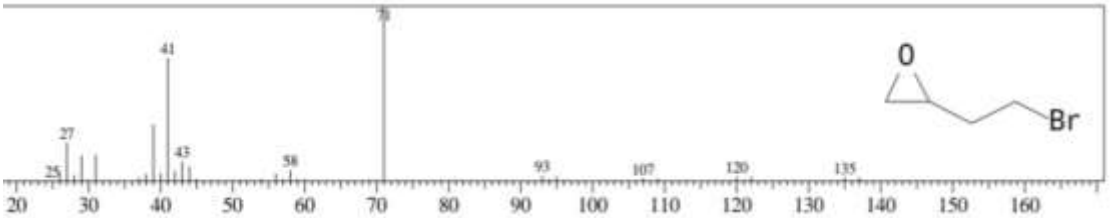

Fig. 29: Structure of oxicirine in roots of *A. maurorum* using GC-MS.

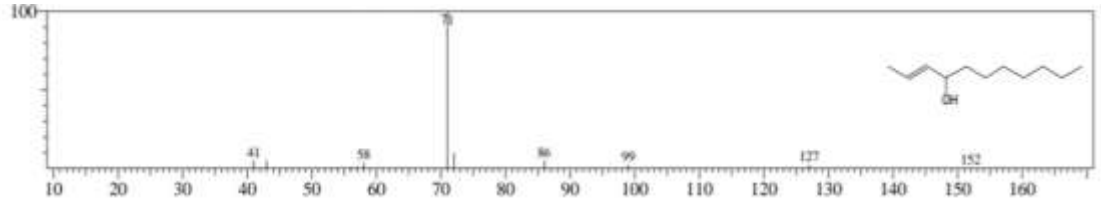

Fig. 30: Structure of 2- undecane -4- ol in roots of *A. maurorum* using GC-MS.

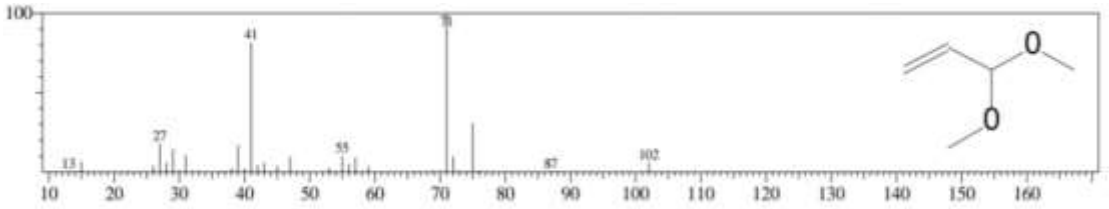

Fig. 31: Structure of acrolein, dimethyl acetal in roots of *A. maurorum* using GC-MS.

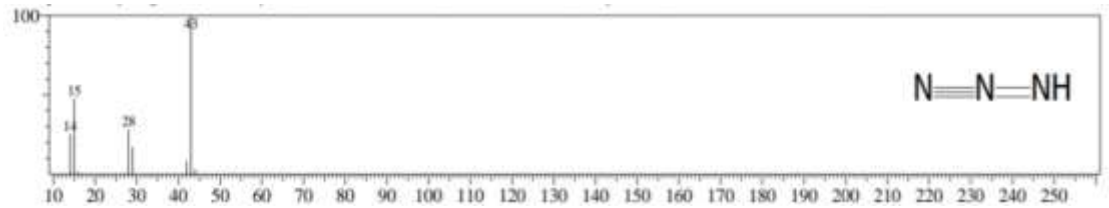

Fig. 32: Structure of hydrogen azide in roots of *A. maurorum* using GC-MS.

The above results of GC-MS reactions showed different constituents of active compounds that are distributed unequally along the peaks; for example, oxalic acid was determined in peak 1 and peak 14, while isobutane was found in peak 2 and peak 6. This unequal distribution is attributed to the difference in their molecular weight (as shown in table 1). The phytochemical compounds listed in the table are the exact reasons related to the medicinal advantages of this plant, such as the antibacterial and anti-inflammatory activities [10]. The results revealed that camel- thorn has many active compounds, including terpenes, fatty acids, phenols, and flavonoids in high concentrations [11]. Soad *et al*.[12] stated that the GC-MS analysis of ethanolic extract of camel thorn roots showed the presence of thirteen compounds. Researchers found that the stem of *A.* pseudalhagi have seven secondary metabolites that are effective against various diseases and have pharmacological importance [13] They are considered as anti-inflammatory [10], anti-cancer [14], antibacterial [15], anti-ulcerogenic [7], anti-fungal [12], cancer preventive [16], and anti-malarial agents [17]. Other researchers used GC-MS analysis to identify various bioactive complexes (phenols, tannins, and fatty acids) in *A. maurorum* that showed insecticidal activity [18].

Conclusions

The current study concludes that *A. maurorum* root extract contains phytochemical compounds which have many biological activities. *A. maurorum* is rich in anticancer compounds and other constituents with many biological activities. The phytochemicals of selected plants must be investigated further to develop them into pharmaceutical drugs.

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