Journal of Kerbala for Agricultural Sciences Issue (2), Volume (8), (2021)

Separation and identification of many volatile oil compounds and phenolic compounds from the seeds of *Ammi visnaga* (L.) growing in Iraq

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Received:

Oct. 17, 2021

Accepted:

Nov. 27, 2021

Published:

Dec. 01, 2021

Abstract

The current research was contained the separation and identification of Many volatile oil compounds from the seeds of *Ammi visnaga* (L.), using converted Clevenger apparatus for night oil and the results were confirmed by using GLC technique and identified of many volatile oil compounds as following: (α- pinene, Linalool, Sabinin, Limonine, Terpinen and Myrcene, and these componds were appeared in the normal water extract and zamzam water extract and also in the pet- ether extract (A1) and in chloroform (A2) and also in Acetone extract (A3) and the higher concentration of these compounds was found in the zamzam water extract. We were obtained many of plant extracts from the seeds of Ammi visnaga (L.), using soxhlet apparatus with following solvents as following: pet. Ether (A1), Chloroform (A2), Acetone (A3), Ethanol (A4), as well as the hot aqueous extract (A5). Moreover, the acid hydrolysis was carried out to get some free phenolic compounds from the (A3, A4 and A5) extracts and identification was investigated by using HPLC technique to appear the following phenolic compounds; (Coumarin, Apignin, Kaempferol, Caffeic acid, Rutin, Quercetin, Visnagin and Ferulic acid). All the phenolic compounds were appeared in the mentioned three extracts except of ferulic acid that was not appear in Acetone extract (A3) and the highest concentration of these compounds was in the hot aqueous extract (A5).

Keyword: *Ammi visnaga*, Furo comarins, Flavonoids, Volatile oils.

Introduction

The Ammi species belong to the family Umbellifereae (Apiciea) and *Ammi visnaga* (L.) is a natural substance long used in herbal medicine and it contains khellin compound that promotes widening of the blood vessels. It has been used to treat conditions ranging from the menstrual cramps to atherosderosis and also some people take ammi visnaga orally and others use it topically to treat certain skin conditions [1].

A. visnaga (L.) contained σ- pyrones (furan chromon up to4%), the priniciple compounds being khellin (0.3- 1.2 %), visnagin (0.05- 0.30 %) khellinol, ammi ol, khellol

and khellinin, ammi visnaga also contained fixed oils up to 18 % and coumarins (0.2-0.5 %) and the main one being the pyranocoumarin visnadin (0-3 %) [2].

The essential oil of fresh aerial parts of *A. visnaga* through different stages of the plant growth collected from Boumerdes (Algeria) obtained by hydro- distillation in Clevenger type apparatus, was analyzed by GC- MS, and the results demonstrated that the best yield was obtained at the fructification period (0.48 %) and the major compounds of *A. visnaga* essential oils at three development stages are firstly the 2- methyl-butyl, 2- methl butanouatc (10.29, 16. 533, 28. 56 %) representing respectively and aslo β- Linalool was presented with (9.18 %) [3].

Phenolic compounds were also presented in the *A. visnaga* as eleven flavonoids have been isolated from the aerial parts of *A. visnaga* from which four aglycones, four mono-glycosides, two diglycosides, and the one triglycoside, also the flavonoid a glycones were distributed in to one hydroxylated (Quercetin) and three methoxylated, the mono-glycosides in cluded three 3-0- glucosides respectively linked to rhamnetin, isorham-netine and rhamnazin and one 7-0- glucoside of Isorhamnetin, the two diglycosides were 3-0- rutin of quercetin and isorhamnetin, while the single triglucoside was quer-cetin; 7, 3, 3¯- 0- triglucosides. Also, the main volatile compounds was identified from the *A. visnaga* It was confirmed two volatile oil compounds, Linalool (24- 8- 44- 2 %) and Limonene (% 9.9 -6.8) [4, 5]

Moreover, it was dealt [6] with the vaiorazation of medicinal plant and aromatic plants of the Tunisian flora in order to find new bioactive natural products, and they were extracted and confirmed forty-one volatile oil compounds and the major com-pounds were linalool, Isovalerate as well as the low concentration of α- pinene, Sab-inene, β- Pinene, Myrcene and α- Terpinen.

Materials and Methods

Collection of the seeds of *A. visnaga*

The *A. visnaga* (L.) seeds were collected from different regions of Mosul city and classified in the Directorate of the medicinal plants Development project in the Mosul Dam of the Iraq ministry of Agriculture and Agricultural Reform. After that, the seeds were cleaned from dust and so on, then they were gowned and put it in paper bags and kept it conditions away from moisture until use.

Classification of the *A. visnaga* (L.)

The plant was classified by Dr. Amer Mohson, the assistant professor in the Biology Dept. in the college of Education and pure science.

Kingdom → Plantae

(IT/ S, 2020 IT/ S, 2020).

Volatile oils extracted by converted Clevenger pivot steam distillation Appa-ratus

Volatile oil compounds were extracted from the seeds of the study plant using aspecilized Clevenger device to extract the light oil and connected with a volumetric

flask with a capacity of 500 ml, as 15 gm of the seeds of *A. visnaga* (L.) as powder was mixed with 200 ml of (d. w) and then the distillation process was carried and with the boiling point 100 ℃ and the process of distillation lasted between (1-2 hrs). Distilled water containing the volatile oil was collected and put it in the separating funnel 100 ml of it and 50 ml of ether was added to it for two stages, shake the mixture well and then left to settle, two layers and concentrated it by using rotary vacuum evaporator. The crude oil was placed in dark bottles and kept in the refrigerator until identified [7]. Also, we used the 200 ml of Zamzam and we are repeated some process to get the volatile oil compounds.

1.Preparing of some plant extracts using continuous soxhlet Apparatus .

The seeds of *A. visnaga* were crushed by an electrical mill, where 25 gm of the ground powder which was placed in the soxhlet batch system and added 400 ml of each solvents as following; pet- ether (60- 80 ℃) (A1), chloroform (A2), Acetone (A3), Ethanol (A4), and also we used the hot aqueous extract (A5). All the plant extracts were concentrated by votary vacuum evaporater to get (20- 25 ml) of the crude extract for each used solvent.

2.Acid hydrolysis process to get free pole of some phenolic compounds from the seeds of *A.visnaga*

A mixture of 10 ml of the crude extracts (A3, A4 and A5) for each one and 25 ml of (1N) HCl was refluxed to 90 min- at 100 ℃, put the solution after cooling in aseparating funnel, after that added (2 × 10 ml) of ethyl acetate. After the isolation of aqueous layer from organic layer, using magnesium sulfate, dried the organic layer and concentrated the ethyl acetate extract using rotary vacuum evaporator. Then sample of separated phenols was kept in glass bottles until the analysis by HPLC [8].

3.Qualitative and Quantitative Determination of volatile oils using GLC- technique for *A. visnaga* (L.).

Chromatographic analysis of the diagrams was investigated in which the retention times of each volatile compound was determined for study sample compared to the authentic sample retention time.The separated volatile compounds were identified in the laboratories of the ministry of science and technology/ Department of Environment and water by GLC model (Shimanezo, Japanese) using ionized flam detector and using the poetic column type (SE- 30), wavelengths (0.25 mm, 0.54, 30 m). the temperature was in the injection area and the detector (330 and 280 ℃) while the column temperature starts from (120- 280 ℃) at rate of 8 ℃/ min. using passive nitrogen gas as a carrier gas at a rate of 100 kp [9]. Phytochemical method 2 and ed., chapman and Hall).

4.Identification of phenolic compounds by HPLC technique

The identification of phenolic compounds was also performed in the laboratories of the ministry of science and technology/ Dept. of Environment and water, by HPLC model (SYKAM) Germany pump model; S 2100 quaternary gradient pump, auto

sampler model: S 200 detector; Ur (S 2340) and column oven model (S 4225). The mobile phase was:

A= (Methanol: D. W: acetic acid (85: 13: 2)

B= (Methanol: D. W: acetic acid (25: 70: 5)

The column is C18- ODS (25 cm * 4.6 mm) and detector UV- 469 nm at flow rate flow 1 ml/ min [10].

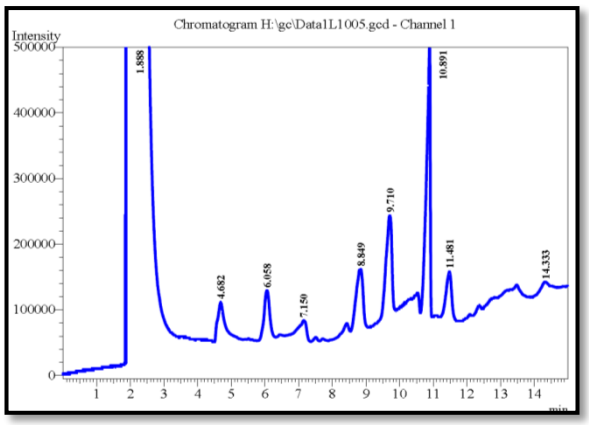
Results and Discussion

1.Chromatographic identification of volatile oils using GLC technique for A. *vis-naga*

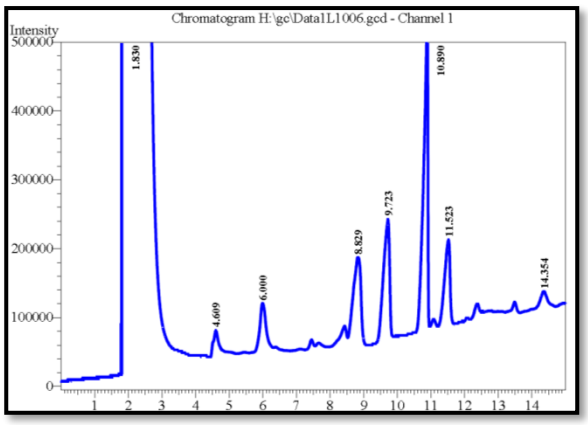
Chromatographic charts were obtained in which the retention time of each compound was determined for study samples compared to the standard sample retention time of α- pinene (11. 54 min)., Linalool (4- 61 min), sabinen (8- 89 min), Limonene (10- 82 min), Terpenin (6- 08 min) and Myrcene (9- 99 min). from the table (1) we noticed that the highest concentration on these compounds was in zamzam water and the percentage ratio for these above-mentioned compounds (20- 5, 150 8, 14- 9, 19- 8, 7- 9, and 5.9 % respectively in the different plant extracts (Normal water, zamzam water, pet- ether extract (A1), chloroform (A2), Acetone extract (A3). (Table 1), and this result was approved with kabouche and Jay

Table (1): The percentage ratio of concentrations of some identified Volatile oil compounds by using GLC technique

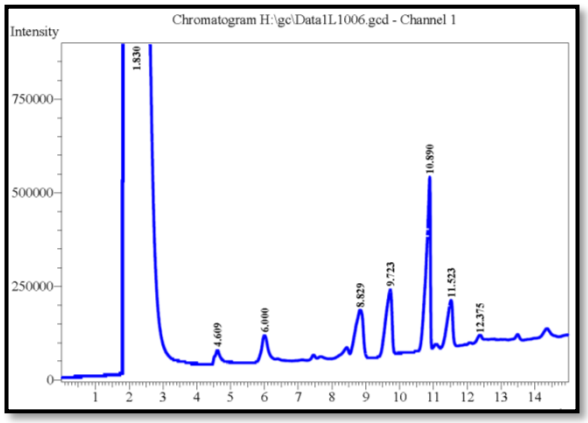
No.	Compounds%	Rt(min.)	Normal water%	Zamzam water%	Pet. Ether extract%	Chlorofrom extract%	Acetone extract %
1	Alpha- pinene	11.549	16.8	20.5	18.4	16.0	15.4
2	Linalool	4.613	13.2	15.8	14.2	12.7	12.2
3	Sabinen	8.895	12.2	14.9	13.0	11.7	11.0
4	Limonene	10.823	16.5	19.8	17.9	16.0	15.3
5	Terpene	6.082	6.4	7.9	7.0	6.0	5.4
6	Myrcene	9.990	4.3	5.9	5.1	4.0	3.6



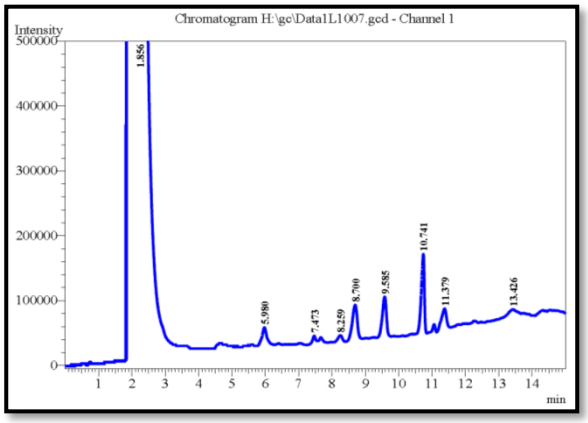
Fig(1) The isolated and identified of Pet.ether extract (A1) from the seeds of *Ammi visnaga (L.)* by GLC technique.



Fig(2) The isolated and identified of Chloroform extract (A2) from the seeds of *Ammi visnaga (L.)* by GLC technique.



Fig(3) The isolated and identified of Acetone extract (A3) from the seeds of *Ammi visnaga (L.)* by GLC technique.



Fig(4) The isolated and identified of Normal water extract from the seeds of *Ammi visnaga (L.)* by GLC technique.

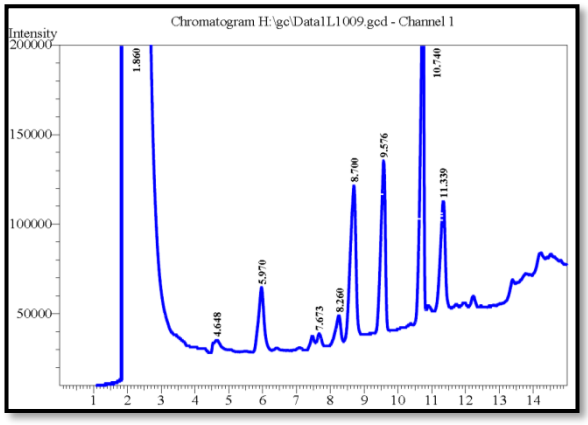
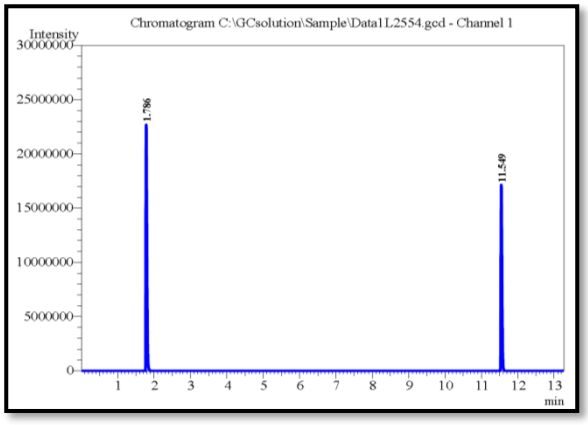
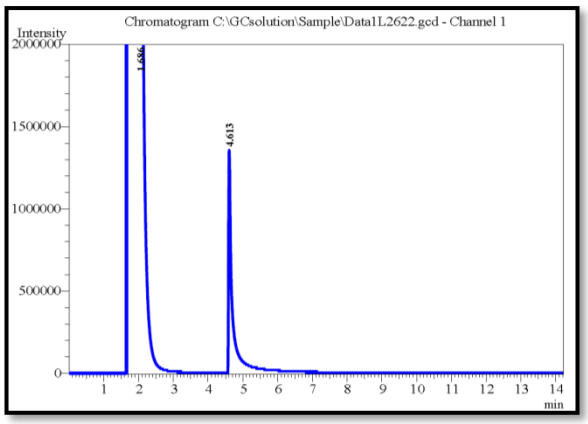


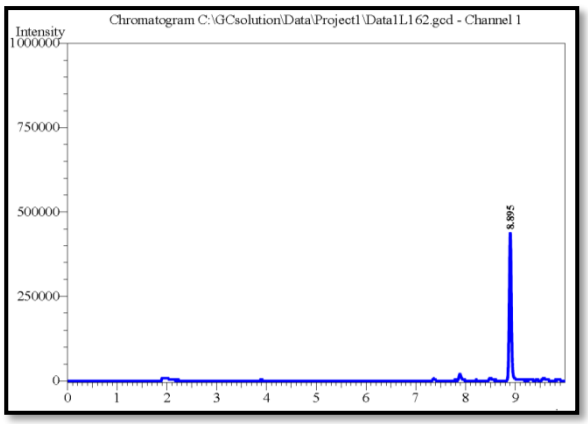
Fig (5) The isolated and identified of Zamzam water extract from the seeds of *Ammi visnaga (L.)* by GLC technique.



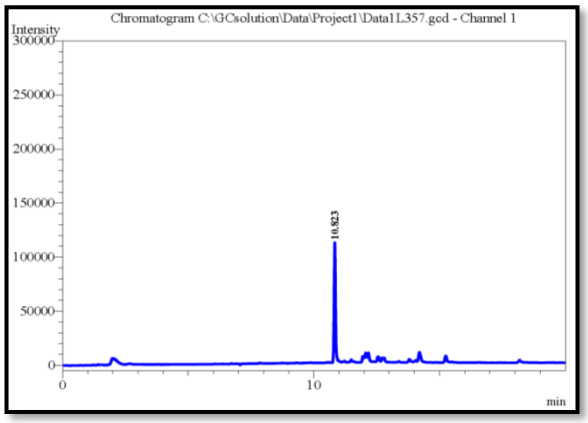
Fig(6) the standared curve of identified α-Pinene by using GLC technique



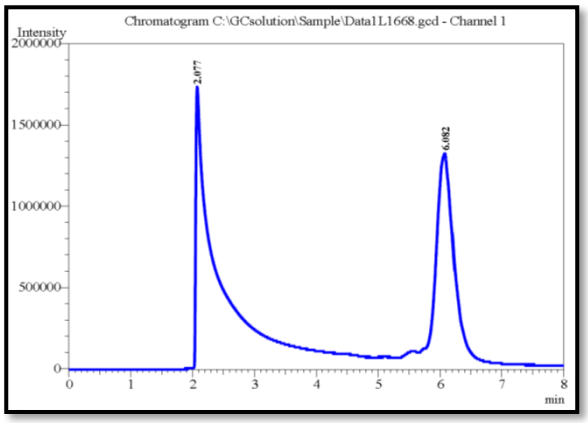
Fig(7) the standared curve of identified Linalool by using GLC technique



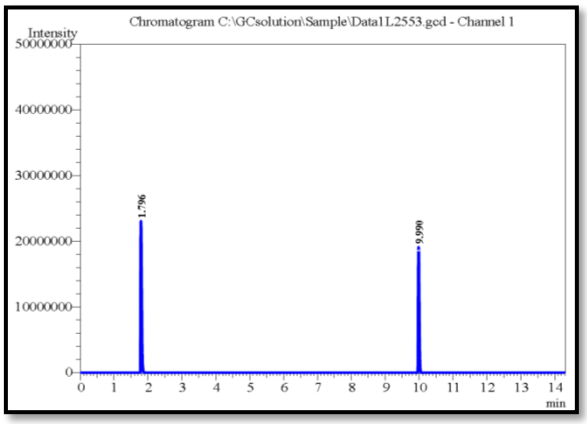
Fig(8) the standared curve of identified Sabinen by using GLC technique



Fig(9) the standared curve of identified Limonene by using GLC technique



Fig(10) the standared curve of identified Terpinoid by using GLC technique



Fig(11) the standared curve of identified Myrcene by using GLC technique

1. Identification of Phenolic compounds by HPLC technique

Analytical charts were obtained and the retention time of each sample was determined for the study sample compaired to the sample time of standard, Coumarin (8.09 min), Apigenin (9.03 min) Quercetin (9- 56 min), Rutin (10- 76), Caffeic acid (11- 33 min), kaempferol (12- 33 min), Visnagin (13- 55 min), and Ferulic acid (14- 19 min), (Table 2). By using calibration curves to calculate the concentrations of the phenolic compounds, and also the concentrations of these compounds from the three extracts; (A3), A4 and A5 after the acid hydrolysis scince it was indicated that the hot aqueous extract (A5) was contained the highest concentrations of phenolic compounds Apigenin (185- 94 µg/ ml), Quercetin (197. 77 µg/ ml), Rutin (114- 89 µg/ ml), Caffeic acid (46. 59 µg/ ml), Keampferol (539. 16 µg/ ml), Visnagin (68. 22 µg/ ml). and Feralilc acid (62. 78 µg/ ml). Also, these results were indicated that the hot aqueous extract as polar solvent to give the high concentration of these phenolic compounds and we found the less concentration of these compounds was in the Ethanol extract and it was also higher polarity and came behind a hot aqueous extract.

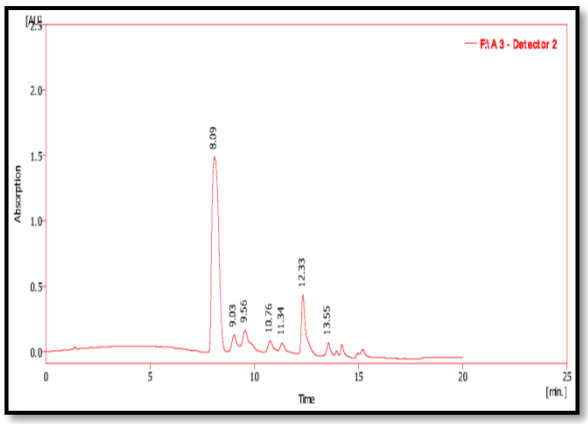
Table (2): The identified phenolic compounds by using HPLC techningue for the Acetone, Ethanol, and hot aquous extracts from the seeds of *A. visnaga* after acid hydrolysis

No.	Standared of phenolic compounds	Rt(min.)	Acetone extract Amount% µg/ml	Ethanol extract Amount% µg/ml	Hot aquous extract Amount% µg/ml
1	Coumarin	8.093	1.445	4.200	6.014
2	Apigenin	9.030	74.202	91.159	185.942
3	Quercetin	9.560	43.202	74.682	197.777
4	Rutin	10.760	32.650	43.163	114.897
5	Caffeic acid	11.337	11.156	24.557	46.598
6	Keampferol	12.333	102	260.666	539.166
7	Visnagin	13.550	22.721	29.873	68.227
8	Ferulic acid	14.193	–	26.015	62.781

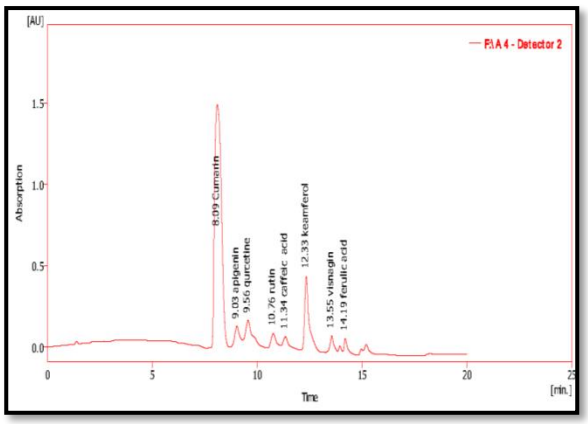
Table (3) :The retention times (Rt) of some identified phenolic compounds by using HPLC technique from the seeds *A. visnaga*

Calibration Summary Table (ESTD - F:\phenolic compound mix - Signal 1)

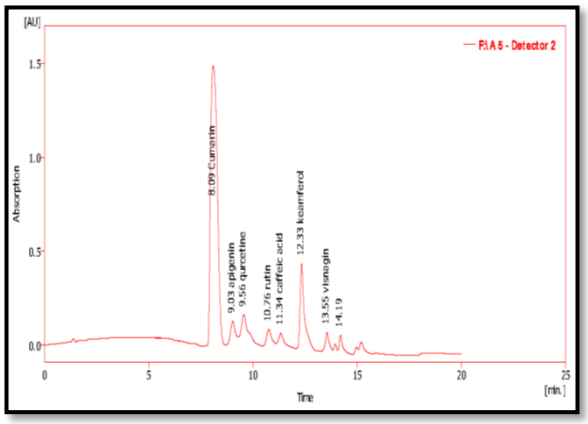
Used	Compound Name	Reten. Time	Left Window	Right Window	Peak Type	Peak Color	LOD	LOQ	RB	Resp. Factor
☒	Cumarin	8.093	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	apigenin	9.030	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	qurcetine	9.560	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	rutin	10.760	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	caffeic acid	11.337	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	keamferol	12.333	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	visnagin	13.550	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
☒	ferulic acid	14.193	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000



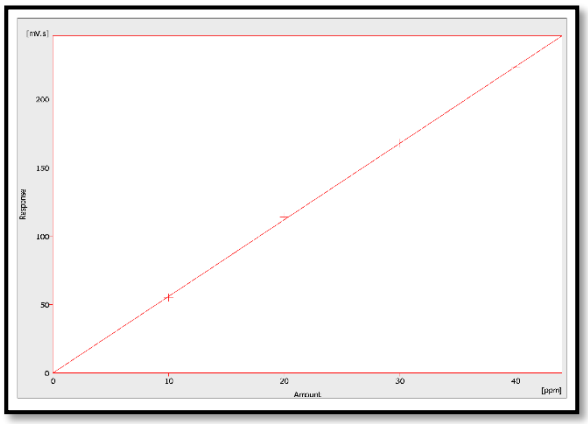
Fig(12) The isolated and identified phenolic compounds from Acetone extract (A3) from the seeds of *Ammi visnaga(L.*) by HPLC technique



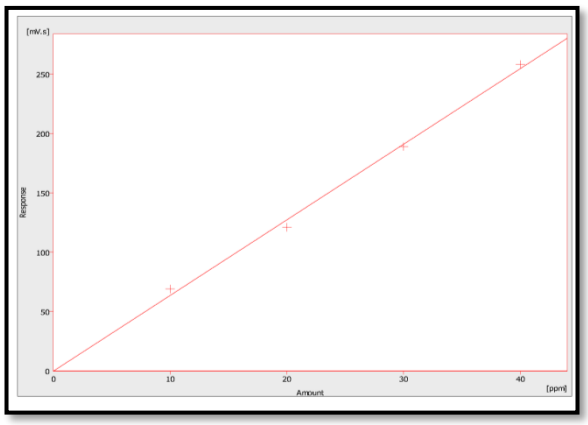
Fig(13) The isolated and identified phenolic compounds from Ethanol extract (A4) from the seeds of *Ammi visnaga(L.)* by HPLC technique



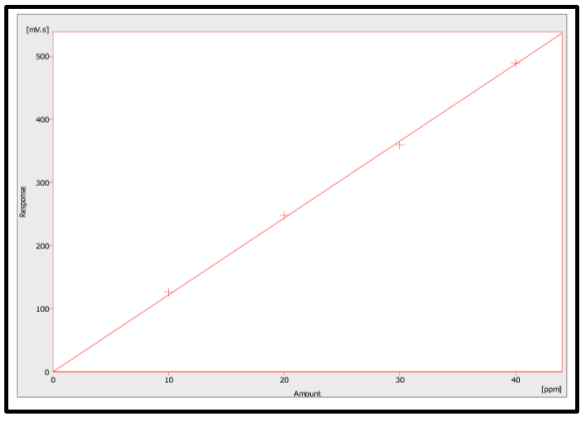
Fig(14) The isolated and identified phenolic compounds from Hot aquous extract (A5) from the seeds of *Ammi visnaga(L.)* by HPLC technique



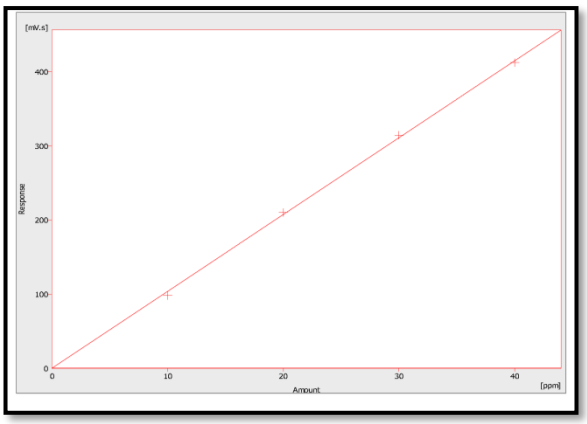
Fig(15) the standard calibration curve of identified Coumarin by HPLC techuique



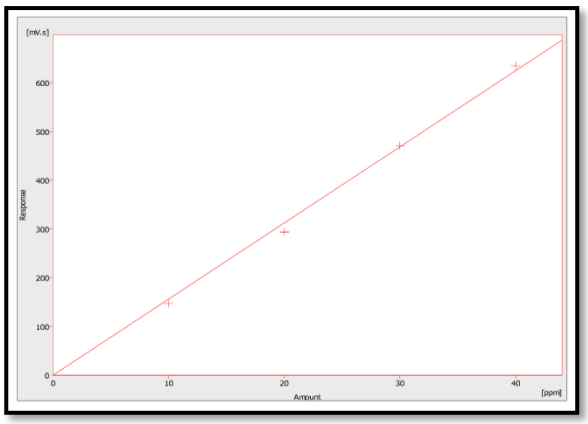
Fig(16) the standard calibration curve of identified Apigenin by HPLC techuique



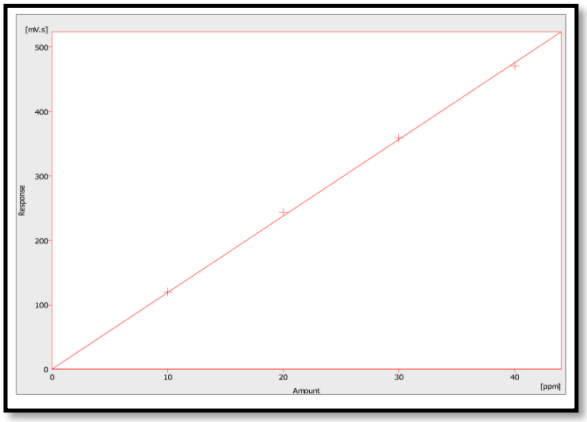
Fig(17) the standard calibration curve of identified qurcetin by HPLC techuique



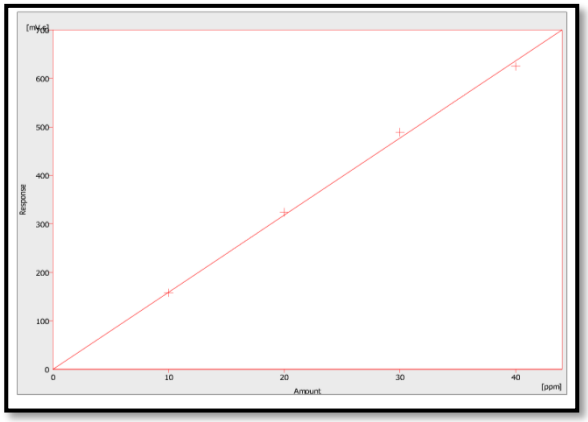
Fig(18) the standard calibration curve of identified Rutin by HPLC techuique



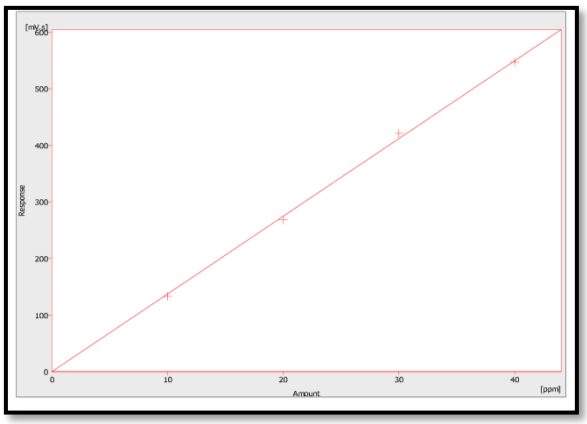
Fig(19) the standard calibration curve of identified Caffeic acid by HPLC techuique



Fig(20) the standard calibration curve of identified Keampferol by HPLC techuique



Fig(21) the standard calibration curve of identified Visnagin by HPLC techuique



Fig(22) the standard calibration curve of identified Ferulic acid by HPLC technique

The volatile oil compounds and phenolics material were showed in various extracts from the seeds of *A. visnaga*, that also growing in Iraq and confirmed by using GLC and HPLC techniques. These mentioned compounds were very important in multiple cases especially in the medicinal treatment.

Acknowledgments

May thanks go to the staph of Department of Biology, College of Education for Girls, University of Mosul for the logistic help it has provided.

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