Qualitative and quantitative separation of some volatile oils and phenolic compounds from the tubers of *Cyperus rotundus* L. plant growing in Iraq

Attaa H. Ghanim [1] , Ayad C. Khorsheed [1]*

[1] Department of Biology, College of Education for Girls University of Mosul, Mosul, Iraq
Corresponding Author email: ayad_chachan@uomosul.edu.iq

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Abstract

The current study aimed to separation and identification of many volatile oil compounds in the tubers of Iraqi *Cyperus rotundus* using clevenger apparatus for light compounds and gas liquid chromatography (GLC) technique. Many volatile oil compounds were identified as following (Limonene, Cymene, Linalool, α- Pinene, Cineol and Terpinen) and the highest concentration was the cineol (58.2%) and lowest concentration was the cymene (0.55%). Also, multiple phenolic compounds were identified in the various extracts including rutin, myricetin, apigenin and gallic acid in the (Cr_3) extract. Furthermore, the Vanillic acid, Ferulic acid, Myricetin, Gallic acid) in the (Cr_4) extract. Moreover, Quercetin, p-coumaric acid, Kaempferol, Myricetin, Tannic acid and Apigenin in both extract (Cr_5) and Zamzam extract (Cr_5).

Keywords: *Cyperus rotundus*, volatile oils, phenolic compounds, Acid hydrolysis

Introduction

Cyperus rotundus (Family cyperaceae), also known as purple mutsedsge, is common perennial weed with sender , scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long, the tubers are externally blackish in colour and raddish white inside with specific odour and the stems grow to about 25 cm tall and the leaves are linear,dark green,and grooved on the upper surface [1].

Volatile oils are complex mixtures, constituted of terpenoid hydro carbons, oxygenated terpenes and sesquaiterpenes, also they orignated from the plant secondary metabolism and are responsible for their specific aroma. All applications of volatile oils may be found in the cosmetic field, as ingredients of fragrances, decorative cosmetic and flavouring,in the food industry , as aromas and flavours in the pharmaceutical industry as active compounds of medicin and as antibacterial /antimicrobials and in the aromatherapy, more over it has been used in the production of lubricants, soap and personal care , product as well as in treatment of various conditions such as hair dandruf, muscle spasms, varicose veins and wounds [2,3].

Also there have been several reports of medicinal plants being utilized as an alternative source of therapy for a variety of pathophysiological diseases, including dibales

and oxidants ,also in Yemen widely grows between khat tree, with edible, fresh roots [4].

The use of this compound in pharmaceutical treatments such as anti- inflammatory and antipyretic therapy has gained popularity[5], antiheumatic antiulceric and anti-neural agent[6], anticarcino genesis effect [7], antibacterial activity[8].

The methanolic extract of the rhizome also shown hepato protoctive and antioxidant activity, as well as a suppression of lipid peroxidation, as well as to other effects [9,10, 11]. The importance of the current research is that it studied for the first time the *C.rotundus* rhizomes extract that grows in Iraq- Mosul and we also investigated the phytochemical screened of *C.rotundus* extract for the detection of active components such as volatile oils, Fatty acids, phenolic compounds and amino-acids by using various chromatographic analysis.

Also, zam zam water refers to one of the important paranormalities in its use, as it is a divine creation and the possibilities of extracting the largest possible number of active substances compared to ordinary water.

Materials and Methods

Plant collection and extract preparation

The rhizomes or tubers of *C.rotundus* were collected from different regions of Mosul city and classified in the Directorate of the medicinal plant project in the Mosul Dam of the Iraq ministry of Agriculture and Agriculture Reform.

Also , the tubers were cleaned from the dust and so on , then they were grown and put it in paper bags (Batch) and kept it conditions away from moisture until use.

The classification of *Cyperus rotundus. L* was carried out by Dr.Amer Mohson . the professor in the Biology Dept. in the college of Education and for science.

Volatile oils extracted by converted Clevenger Apparatus [12]

The volatile oil compounds was extracted from the tubers of the study plant, using celvenger device to extract the light oil and conected with a volumetric flast with a capacity of 500ml and used 15gm of the seeds of *Cyperus rotundus .L* as powder was mixed with 200ml of (D.W)and then the distillation process was carried out with the boiling point 100°c and the process of distillation lasted between (1– 2hrs.) The distilled water was put in separating funnel (100ml) and 50ml of ether was added to it for two stages, shaking the mixture well and then left to settle, two layer and concentrated it by using rotary vacuum evaporator. The crude oil was placed in the bottle and kept in the refrigerator until it used and identified.

Preparation of some plant extracts by using a sequence of solvents system extraction, using soxhlet apparatus

The tubers of *Cyperus rotundus L.* were crusherd by an electrical mill , where 25gm of the ground powder which was placed in the soxhlet device to obtain the batch system and added 400ml of each solvents as followings :pet-ether (60 -80)°c

(Cr_1)chloroform (Cr_2) , Aceton (Cr_3) ,IMS (Cr_4), we used the hot water as a queouse extract (Cr_5) and hot aqueouse zamzam extract (Cr_5 zamzam).
All the fourth extract were concentrated by rotary vacuum evaporator to get nearly (25) ml also the hot aqueous extract and hot aqueouse zamzam.

Acid hydrolysis process to obtain the free pool of some phenolic compounds from the tubers of *C. rotundus*

A mixture of 10ml of the crude extracts (Cr_3 ,Cr_4 and Cr_5) for each of them and added 25 ml of (1 N) Hcl was refluxed to 60 min. at 100 °c . After cooling .we put the solution in separating funnel and we added (2*25 ml) of ethyl acetate . After the isolation of aqueous layer from organic layer, using anhydrous $MgSO_4$ for draying the organic layer and concentrated the ethyl acetate extract using rotary vacuum evaporator. The sample was kept in glass bottles until the analysis by HPLC techniquc [13,14].

Chromatographic determination of volatile oils using GLC- analysis from the tubers of *C. rotundus*

Chromatographic analysis of the diagrams were investigated in which the retention time of each volatile oil component was determined for study sample that compaired to the authentic sample retention time .The separated volatile oil compounds were identified in the laboratories of the ministry of Science and Technology / Dept. of Enviroroment and water by GLC model Shimanezo, Japanese , 2010 using ionized flame detector and using the injection area and the detector (280 and 330 °c) while the column temperature starts from (120- 280 °c) at rate of 8°c / min. using passive nitrogen gas as a carrier gas at a rate of 100 kp. Table (1) figs. (1, 2 ,3, 4,5,6,7).

Investigation of phenolic compounds by using HPLC-technique

The identification of phenolic compounds was also carried out in the laboratories of the ministry of Science and Technology / Dept. Enviroment and water by HPLC , model(sykam) Germinary pump model : S 2100 quatermary gradient pump ,auto sample model : S 200 detector, ur (S 2340) and column oven model (S 4225) . The mobile phase was :

A= (Mathanol : D . W : Acetic acid (85:13:2)
B= (Methanol : D . W : Acetic acid (25:70:5)

Also the column is C18-ODS (25cm*4.6 mm) and the detector UV – 469 nm at flow rate flow 1ml/min [15].
Table (2) , Figures. (8,9,10,11,12,13,14,15,16,17,18,19,20,21) .
1-Chromatographic determination of volatile oil compounds using GLC analysis for the tubers of *cyperus rotundus (L)* [16].
Chromatographic analysis of the charts were obtained in which the retention time of each compound was conducted for the study samples compaired to the standard

sample retention of Limonene (5.9 / min.), cymene (1035 min.) Linalool(7.67min .), α-pinene (5.05 min.), cineol (3.39 min.) and Terpinen (4.53 min.). Also , the concentration of these identified volatile compounds were carried out by using the percentage ratio . The highest concentration was found in cineol compound and the lowest concentration was found in cymene compound .Table (1), Figs(1,2,3,4,5,6,7).

2-Identification of phenolic compounds by using HPLC analysis

The analysis of the chromatographic charts were obtained by using the retention time of each sample was determined for the study sample compaired to the sample time of standard compound and we used four extracts; (Aceton (Cr_3), IMS(Cr_4), Hot equeous extract (Cr_5) and Hot aqueous zamzam extract (Cr_5. zamzam).

Gallic acid was appeared with (9.56 min.) and the concentration was 0.0725 mg/gm in Acetone extract (Cr_3), and appeared with (9.55min.)and the concentration was 0.186 mg/gm in the IMS extract(Cr_4).

Also myricetin was appeared with (12.85 min.) and the concentration was 0.066 mg/gm in the Cr_3 extract. Moreover , it was appeared with (12.79 min.) and the concentration was 0.044 mg/gm in IMS extract.

Rutin was showed with (15.02 min.) and concentration was 0.068 mg/gm in the (Cr_3) extract .

Apigenin was showed at (19.78 min .) and the concentration with 0.102 mg/gm in the (Cr_3) extract.

Also, Apigenin was showed with (19.11 min.) and (19.13 min.) with concentration of 0.057 mg/gm and 0.068 mg/gm in Cr_5 & zamzam extract (Cr_5) respectively.

Vanillinc acid was investigated with (3.69 min.) and concentration was 0.136 mg/gm in the (Cr_4)extract. The Ferulic acid was also showed at (6.0 min.) and concentration of 0.075mg/gm in the (Cr_4) extract.

Quercetin was appeared at (5.05 min. and 5.11 min.) and the concentrations were 0.011mg/gm and 0.020mg/gm in the Cr_5 and zamzam extract (Cr_5) respectively .

P.coumaric acid was showed at (8.55 min.) and (8.59min.) and the concentrations were 0.011mg/gm and 0.015mg/gm in the (Cr_5)and zamzam extract(Cr_5) respectively .

Kaempferol also showed at 11.25 min. and (11.30 min.) and concentrations were 0.035 mg/gm and 0.046mg/gm in the Cr_5 extract and zamzam extract respectively.

Finally ,tannic acid was showed at (17.45 min.) and (17.49 min.) and the concetrations were 0.025mg/gm and 0.032mg/gm in the (Cr_5)extract and zamzam (Cr_5 extract respectively. Table (2) , Figures (8,9,10,11,12,13,14,15,16,17,18,19,20,21).

Table (1): The concentration (%) of volatile oil compounds by using GLC technique of normal aqueous extract from the tubers of *C. rotundus*

No	Compounds	Rt(min.)	Normal aq. extract (%)
1	Limonene	5.91	0.14
2	Cymene	10.35	0.55
3	Linalool	7.67	1.05
4	α-pinene	5.05	3.8
5	Cineol	3.39	58.2
6	Terpinen	4.53	4.6

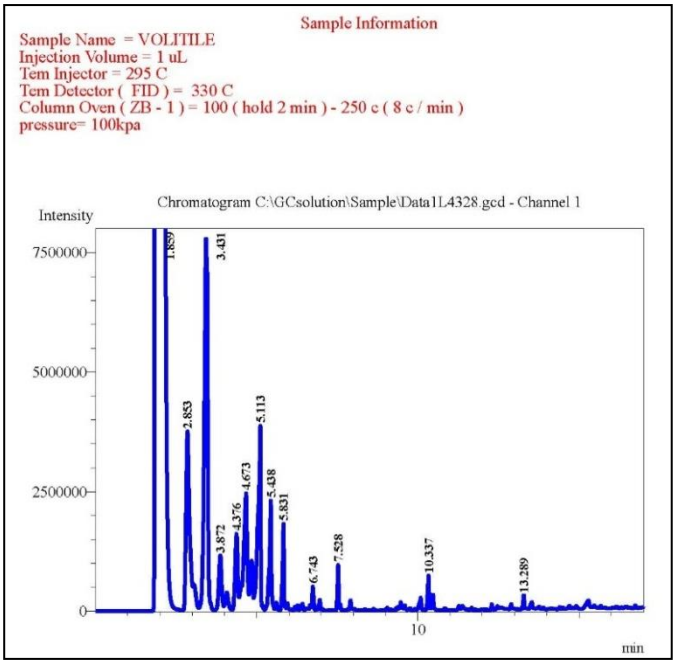

Figure (1): The volatile oil compounds by the Clevenger apparatus

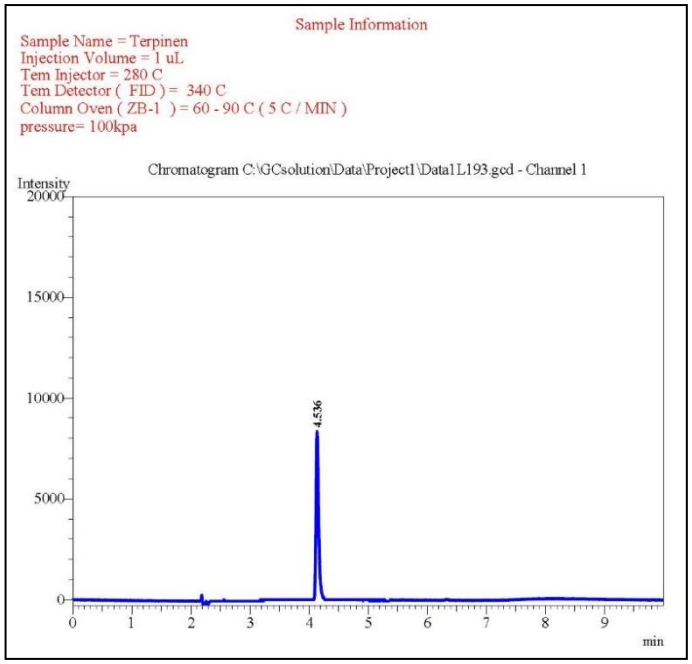

Figure (2): The standard curve of Terpinen

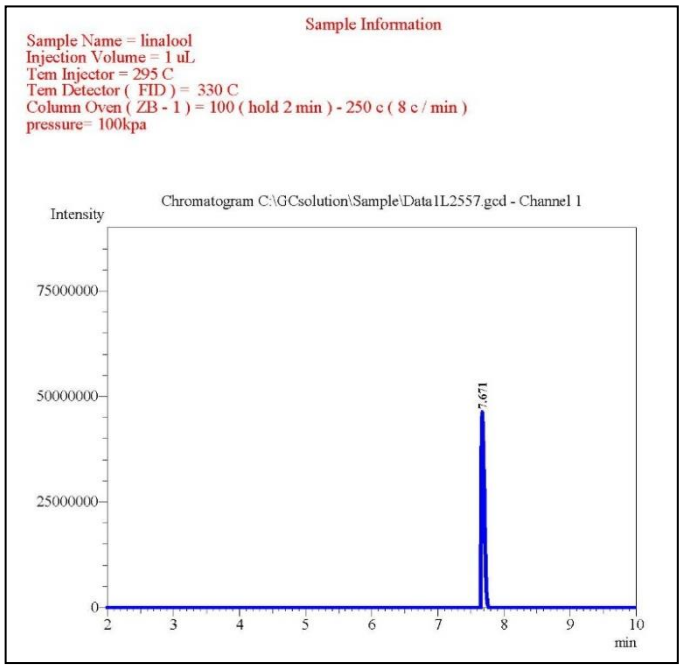

Figure (3): The standard curve of linalool

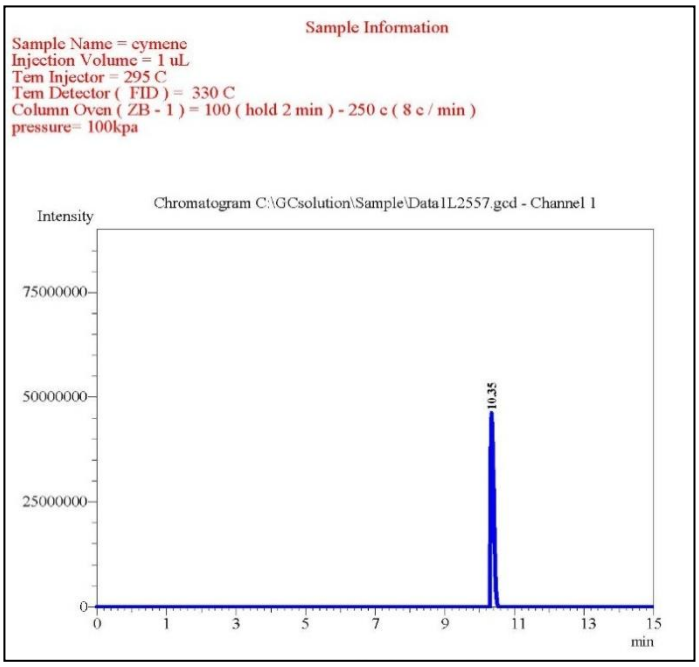

Figure (4): The standard curve of Cymene

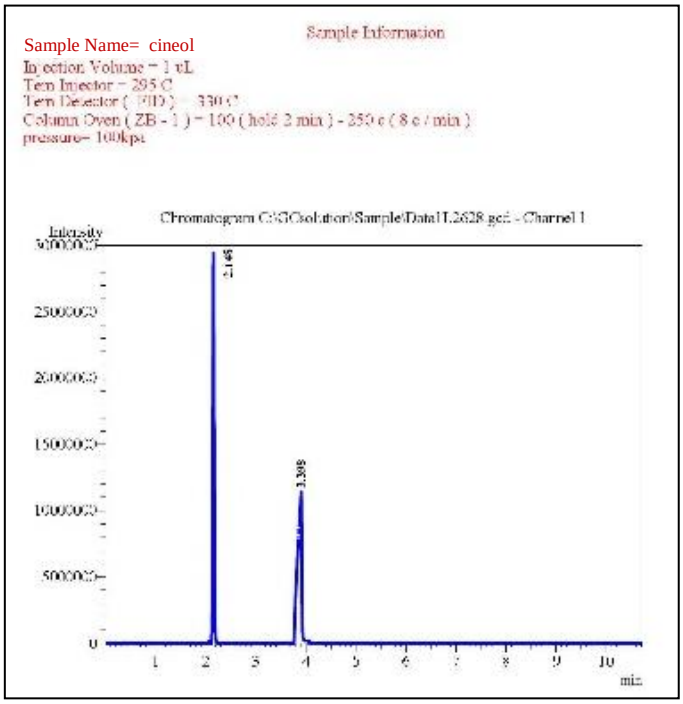

Figure (5): The standard curve of cineol

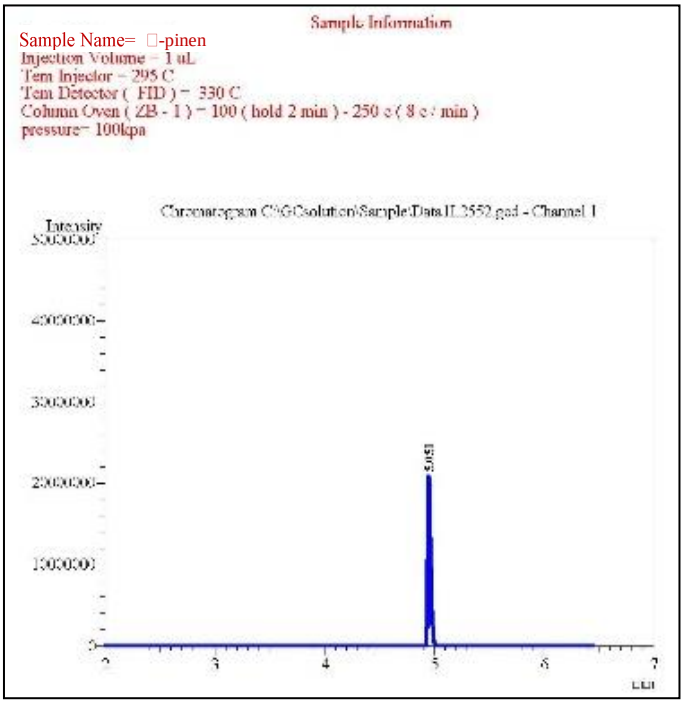

Figure (6): The standard curve of α-pinene

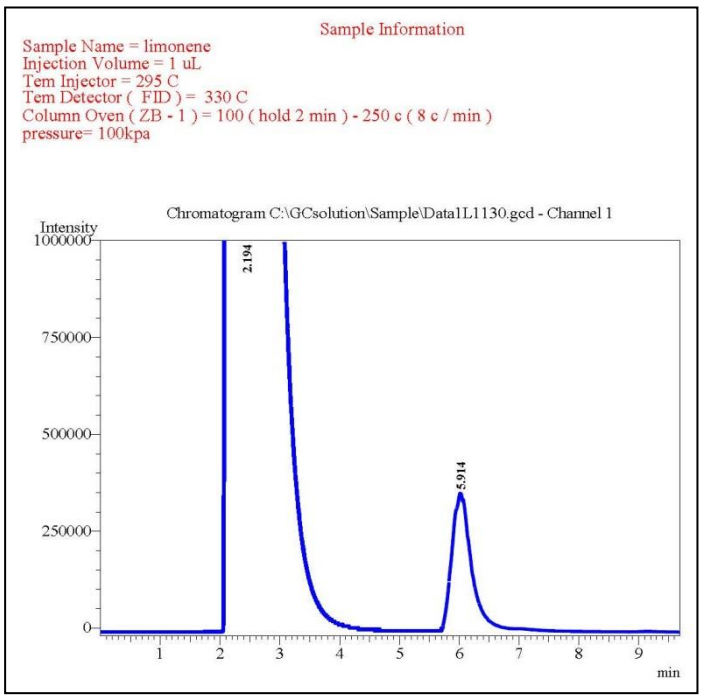

Figure (7): The standard curve of limonene

Table (2) Indicated the standard retention times and the concentration of some phenolic compounds by using HPLC technique of *cyperuse rotundus L.* tubers.

Standard phenolic compounds	Standard Retention times mint.	Aceton extract Cr3		IMS extract Cr4		Hot equeous extract Cr5		Zamzam water extract Cr5	
		Conc. Mg/gm	Rt. Mint.	Conc. Mg/gm	Rt. Mint.	Conc. Mg/gm	Rt. Mint.	Conc. Mg/gm	Rt. Mint.
Gallic acid	9.52	0.07252	9.56	0.186016	9.55	…..	…..	…..	…..
Myricetin	12.85	0.066552	12.85	0.044768	12.79	0.003136	12.79	0.006432	12.85
Rutin	15.00	0.068872	15.02	…..	…..	…..	…..	…..	…..
Apigenin	19.08	0.102088	19.18	…..	…..	0.057576	19.11	0.068904	19.13
Vanillic acid	3.65	…..	…..	0.136544	3.69	…..	…..	…..	…..
Ferulic acid	6.00	…..	…..	0.075536	6.00	…..	…..	…..	…..
Qurcetine	5.00	…..	…..	…..	…..	0.011544	5.05	0.020256	5.11
P.coumaric acid	8.69	…..	…..	…..	…..	0.011264	8.55	0.015344	8.59
Kaempferol	11.10	…..	…..	…..	…..	0.03508	11.25	0.046872	11.30
Tannic acid	17.48	…..	…..	…..	…..	0.02512	17.45	0.032336	17.49

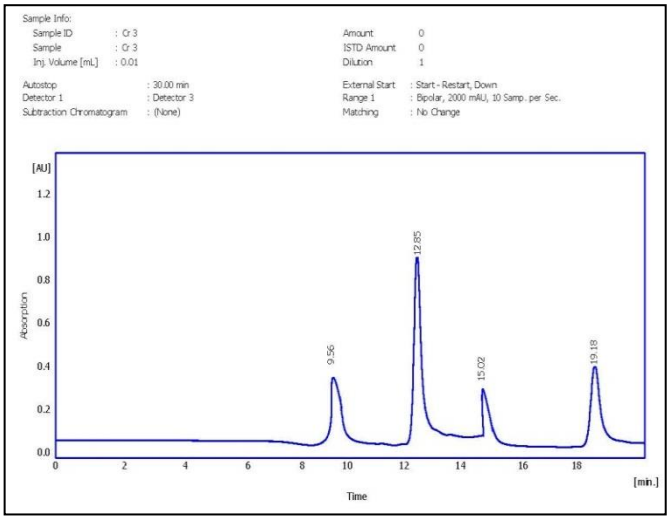

Figure (8): The phenolic compounds form the acid hydrolysis Aceton extract (Cr_3)

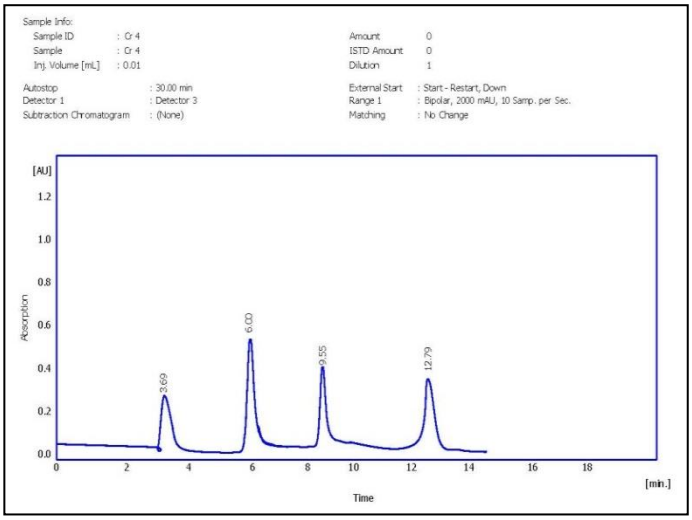

Figure (8): The phenolic compounds form the acid hydrolysis IMS extract (Cr_4)

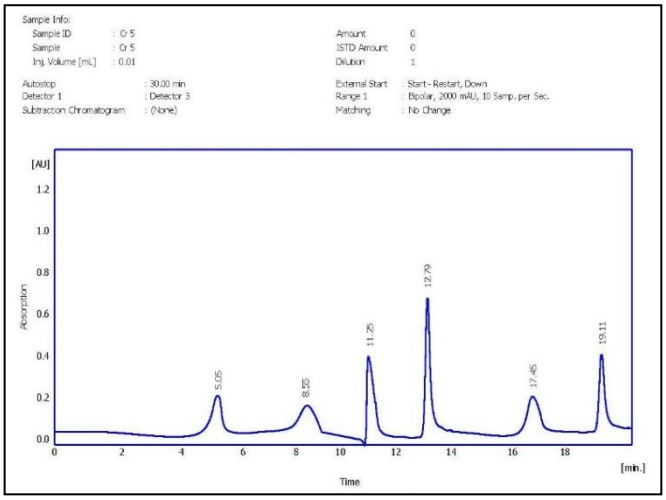

Figure (10): The phenolic compounds form the acid hydrolysis hot aqueous extract (Cr_5)

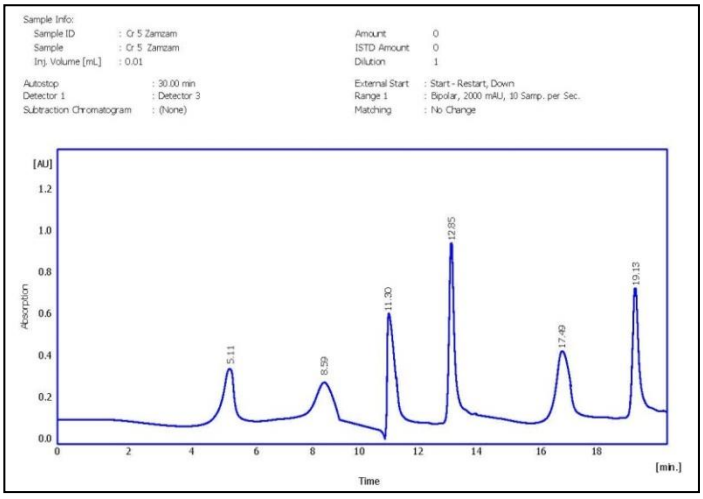

Figure (11): The phenolic compounds form the acid hydrolysis Zam Zam water (Cr_5)

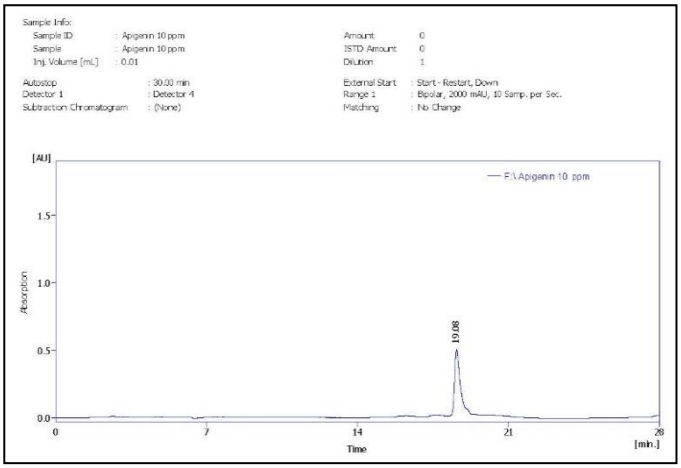

Figure (12): The standard Curve of Apigenin

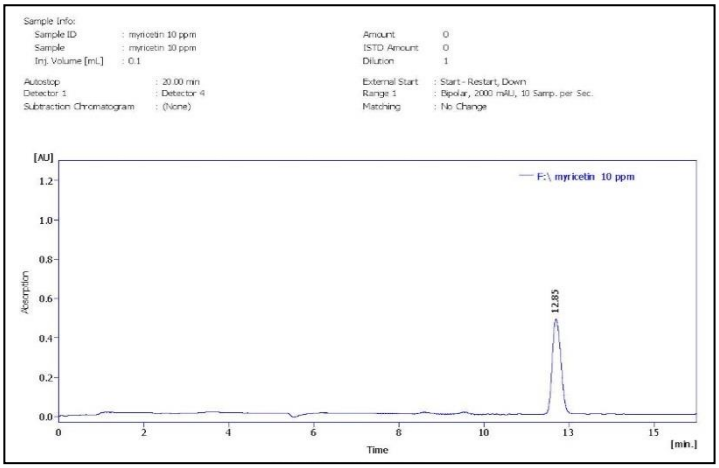

Figure (13): The standard Curve of Myricetine

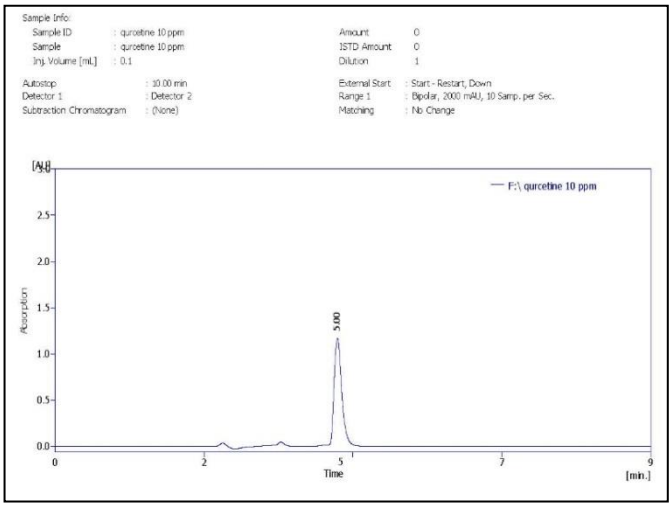

Figure (14): The standard Curve of qurcetine

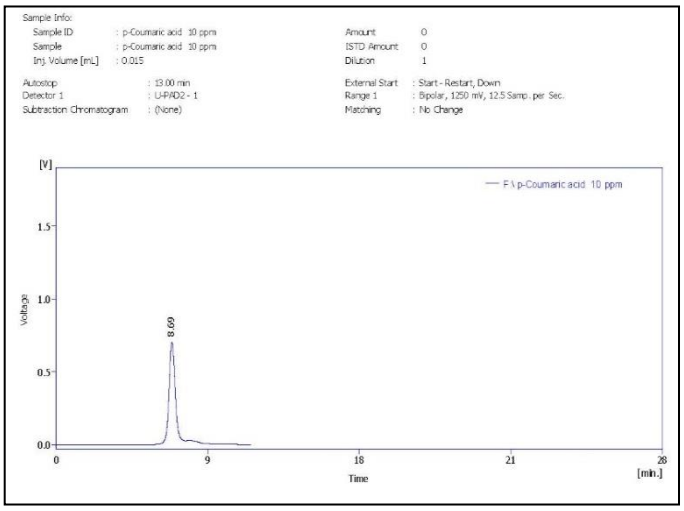

Figure (15): The standard Curve of p-coumaric acid

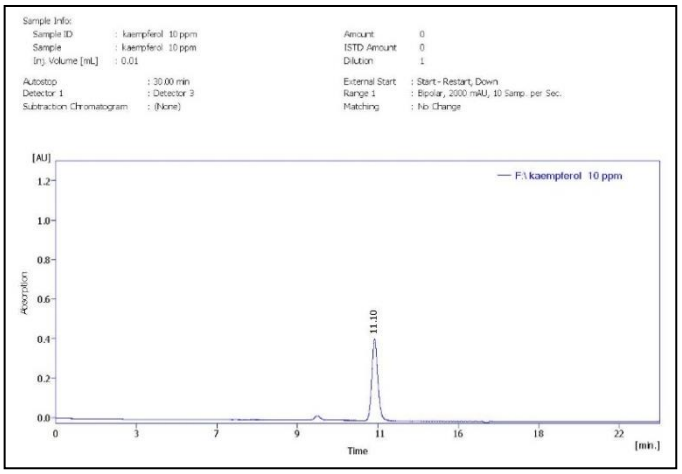

Figure (16): The standard Curve of Kaempferol

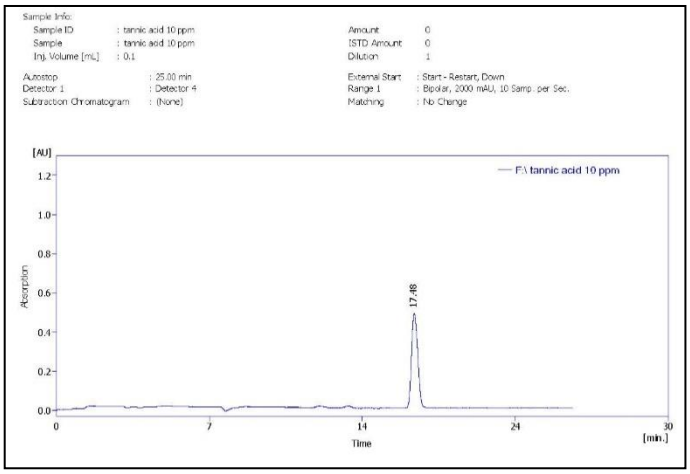

Figure (17): The standard Curve of tannic acid

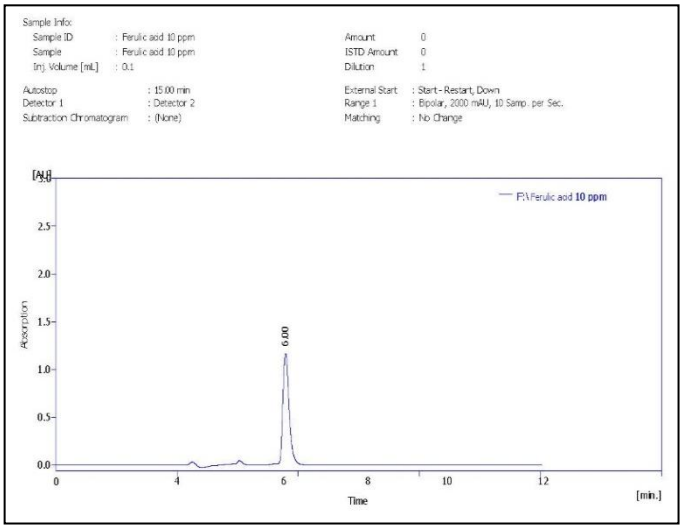

Figure (18): The standard Curve of Ferulic acid

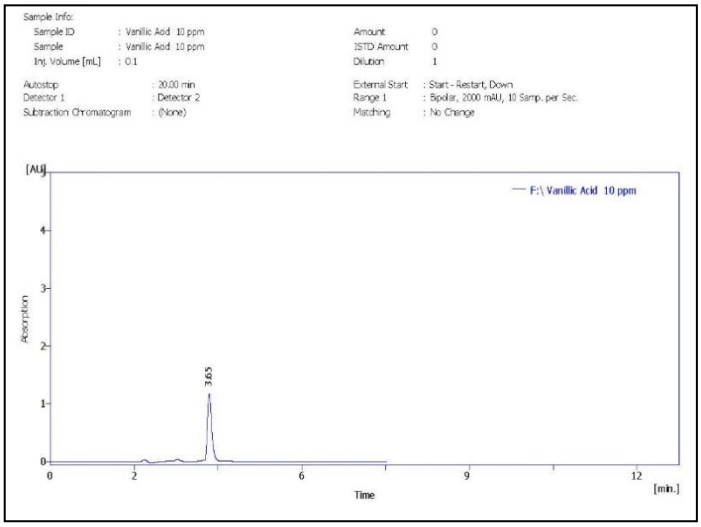

Figure (19): The standard Curve of Vanillic acid

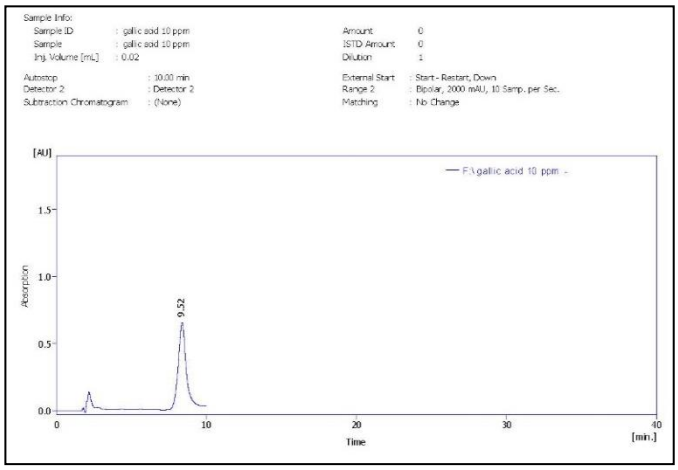

Figure (20): The standard Curve of gallic acid

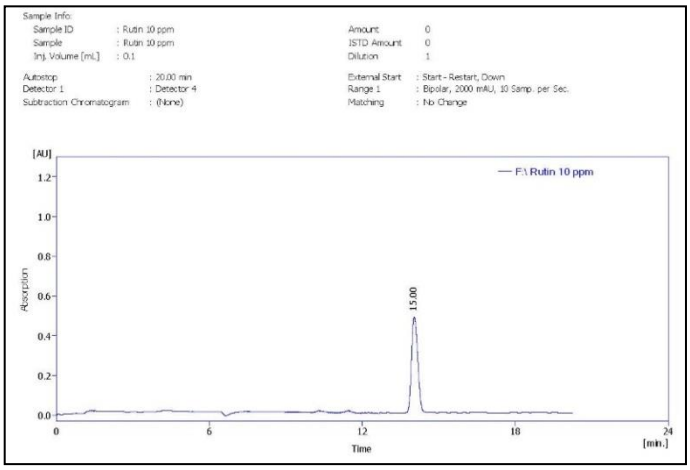

Figure (21): The standard Curve of Rutin

The volatile oils and phenolic compound were showed in different extracts from the tubers of *C. rotundus (L.,)* which is growing in Iraq and investigated by using chromatographic analysis with GLC and HPLC techniques. All compounds that were showed in the various extracts of *cyperus rotundus (L.,)* were important for multiple cases especially in the medical treatment.

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