Evaluation of Phenolic Compounds in Several Species of Some Genera of the Tribe (Poeae) in Iraq and Their Taxonomic Significance

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الخلاصة

ان التشابه والاختلاف في الصفات المظهرية لايدل على ان الوحدات التصنيفية تربط بينها علاقات وثيقة او تعود الى نفس الوحدة التصنيفية . استخدمت تقنية High-performance liquid chromatography (HPLC) لفحص عشرة انواع تابعة لخمسة اجناس للعشيرة Poeae في العـــــراق وهي (Cutandia memphetica, Cutandia dichotoma, Dactylis glomerata, Echinaria capitata, Eremopoa persica, Poa angustifolia, Poa annua, Poa pratensis, Poa triviales and Psilurus incurvus) ؛ حيث تم عزل وتشخيص ثمان مركبات فينولية، وقد بينت النتائج ان بعض الانواع تظهر تغايرات واضحة في تراكيز المركبات الفينولية وبعضها تتشابه تقريبا في محتواها من هذه المركبات ماعدا الاجناس Cutandia dichotoma, Eremopoa persica and Poa pratensis التي امتازت بفقدانها واحد اواكثر من هذه المركبات . ان الهدف من هذه الدراسة هو اثبات ان هذه الانواع لها نفس الاصل وتعود الى نفس العائلة Poaceae والعشيرة Poeae في العراق، وقد تم الاعتماد على المعايير القياسية التي تم الحصول عليها من شركة الحقول البيضاء للاستشارات الهندسية والكيمياوية في بغداد.

Abstract

Since every taxonomist is aware of the fact that morphological similarities and differences of plants does not always indicate close relationship or often belongs to different taxa. High-performance liquid chromatography (HPLC) method has been used for the examination of ten species of five genera of the tribe Poeae which is (Cutandia dichotoma، Cutandia memphetica, Dactylis glomerata, Echinaria capitata, Eremopoa persica, Poa angustifolia, Poa annua, Poa pratensis, Poa triviales and Psilurus incurvus); eight compounds were isolated and identified. The results showed variation in compounds concentration, most species are similar in Phenolic compound composition except Cutandia dichotoma, Eremopoa persica and Poa pratensis which are lacking one or more compounds. The aim of this study is to clarify and improve that these species have the same ancestor and belong to the same family (Poaceae) and tribe (Poeae) in Iraq, depending on the standard values available in the white field company for chemical and engineering consulting in Baghdad.

Keywords: *Plants, HPLC, Tribe Poeae, Phenolic Compound*

Introduction

In the ancient of the time man has been practicing chemotaxonomy of plants by using several senses such as: taste, smell and coloring of the plant organs to gather information about its components and classifying these plants into different categories according to useful and harms characters, it can now be stated unequivocally that the ability to classify is shared by many organisms, particularly in relation to selection of plant parts for feeding and reproduction (6).

The presence or absence of a certain chemical compounds in a certain plant could not be important than the morphological characters such as the presence or absence of petals or the connation of the floral organs (2). The discussion of Plants phenolic is a discussion of plant diversity itself. Although the bulk of these compounds assumed cell wall structure roles (5), the wide spread occurrence of this type of secondary metabolic in Poaceae and currently also in other plant families makes them useful markers for botanical and evolutionary relationships (10). Phenolic compounds are a class of chemical compounds consisting of a hydroxyl group (-OH) bounded directly to an aromatic ring (Hydrocarbon group), these compounds can also be classified as simple phenols or poly phenols depending on how many aromatic rings (12), the species and genera represented in this paper (*Cutandia dichotoma, Cutandia memphetica, Dactylis glomerata, Echinaria capitata, Eremopoa persica, Poa angustifolia, Poa annua, Poa pratensis, Poa triviales* and *Psilurus incurvus*) belongs to the family Poaceae (True Grasses) which are the fifth largest plant family (13) especially the tribe (Poeae), (4) mentions that this family is a very large cosmopolitan consist of 40 tribes , 700 genera and 12000 species, this family is the most important group of useful plants because its species contains bioactive components including phenolic compounds (e.g. Chlorogenic acid, homoorientin, myriscetin, kaempferol, anthraquinon, rutin, quercetin and luteolin) .

Several species of the grasses have been proved to show therapeutic effect (e.g. strong antioxidant properties) (9) and have been effective in the treatment of inflammations and sclerosis (1,11), however it's important to prove that this effectiveness of grasses is belongs to its contents of phenolic compounds. Since the content of these compounds in selected grasses species and genera has not been investigated yet, therefore testing its concentration is of interest, primarily in order to find new source of natural phenolic compounds (8,15) and

their taxonomic significance to separate and recognition of the previous species and genera mention above .

The aim of this study was to investigate the concentration of phenolic compound in the aerial parts of selected species and genera by using HPLC techniques (7), tables and peaks shows the exacts concentration of these compounds.

Materials and Methods

Plant material

The aerial parts of the selected species and genera (leaves and spikes) in the floral stage were collected from the northern side of Iraqi lands which harvested in May 2012, 2013, the herbs were dried at room temperature in special paper.

Determination of Total Phenolic Compounds

Total concentration and content of phenolic compound in the selected taxa extracts were determined by Harborn methods (1973)(15) and the separation occurred on liquid chromatography Shimadzu 2010 LC equipped with binary delivery pump model 2010 (14), the eluted peaks were monitored by UV-Vis 2010 SPD spectrophotometer, and recorded by chromatopack C_R6A integreater Shimadzu, Japan.

Extraction of Phenolic Compounds

The aqueous extract of dried samples were obtained by using boiling water 200ml which was poured over the 1gm of the powder, the mixture was placed in an ultrasonic bath for 15min. and filtered through filter paper (Whatman No.1).

After filtration 100ml of aqueous extract were acidified with 0.5ml of 98% of formic acid to PH=3 and applied to the Octadecyl column (2*5 cm) which was then washed with 100ml of ethanol, then the elute was evaporated using (Buchi system, Germany) to dryness and redissolve in 1ml of the mobile phase to obtained 1ml of the final extracted, 20µl were subjected to HPLC analysis. (Note: Number of dilution times is 25).

Mobile phase were (0.1% Phosphoric acid in Dionized water, Solvent A and acetonitrile Solvent B), Linear gradients from (0 – 100% B) in 10min. The detection UV set at 280 nm, flow rate (1.0 ml/min.), temperature 25ºC. The total content of phenolic compounds in each plant extract was calculated as follows:

$$X = (S1 / S2) * X1 * Nd$$

Where is:

X = Unknown material concentration

S1 = Space pack material

S2 = Space pack measurement

X1 = Standard concentration

Nd = Number of dilution time

Results and Discussion

The total content of phenolic compounds in some taxa of family Poaceae (*Cutandia dichotoma, Cutandia memphetica, Dactylis glomerata, Echinaria capitata, Eremopoa persica, Poa angustifolia, Poa annua, Poa pratensis, Poa triviales* and *Psilurus incurvus*) is mentioned in table 3, while the standard space pack measurement and space pack materials of phenolic compound was mentioned in Table (1,2). Since the phenolic compound played a major role as free radical scavengers (Antioxidant) it was essential to determine their total amount in the selected plant extracts per mg/gm, table 4. Shows that *Eremopoa persica* did not contain any phenolic compound , *Cutandia dichotoma* have only seven phenolic compounds and *Poa pratensis* have six of this compound while other species contain eight phenolic compounds with different concentration varies from small amount of Kaempferol (8.18)mg/gm in *Dactylis glomerata* to (18.85)mg/gm in *Poa pratensis* ,*Cutandia dichotoma* contain high concentration of Chlorogenic acid and Luteolin (2.221.91 , 1.191.91) mg/gm respectively and the compound Rutin was absent in this species which also contain small concentration of Myriscetin (97.76) mg/gm, and almost significant concentration of Quercetin (713.35) mg/gm, this species can be separated from other species of genus *Cutandia* (*Cutandia memphetica*) by the presence of Rutin in the latest species with concentration (379.85) mg/gm, and low concentration of Homoorientin. It is obviously clear that the lowest concentration of all phenolic compounds was in *Dactylis glomerata* except of Rutin and Quercetin which show high concentration (301.97, 530.49) mg/gm respectively. *Echinaria capitata* show high concentration of Homoorientin (569.52) mg/gm and Luteolin (898.96) mg/gm, but still lower in its concentration from *Cutandia* species. *Poa* species (*Poa angustifolia, Poa annua, Poa pratensis and Poa triviales*) can be separated taxonomically by

the variance of its phenolic compounds, *Poa pratensis* lacking two compounds Homoorientin and Myriscetin, and the highest concentration of these two compounds was in *Poa annua* (117.98 and 57.51) mg/gm respectively, this previous species has also high concentration of Kaempferol (290.72) mg/gm and Quercetin (277.02) mg/gm in comparative with other *Poa* species. *Poa pratensis* showed significant concentration of Luteolin (338.21) mg/gm, while the highest concentration of Anthraquinon and Rutin was in *Poa angustifolia* with concentration (176.88 and 325.49) mg/gm. Finally *Psilurus incurvus* has (353.81) mg/gm of Chlorogenic acid and (874.54) mg/gm of Quercetin and low concentration (43.39) gm/mg of Anthraquinon table 4.

(3) Mentioned that *Dactylis glomerata* L. is a much differentiated species in its both morphological and physiological traits. It posses different economic values not only according to isoenzyme and flavonoids polymorphism, but also according to phenolic profiles.

As mentioned above phenolic compound was first diagnosed in this paper in the selected taxa played major role as taxonomic evidence which support to classify these taxa into the exact species and genera and this study was one of the kinds here in Iraq.

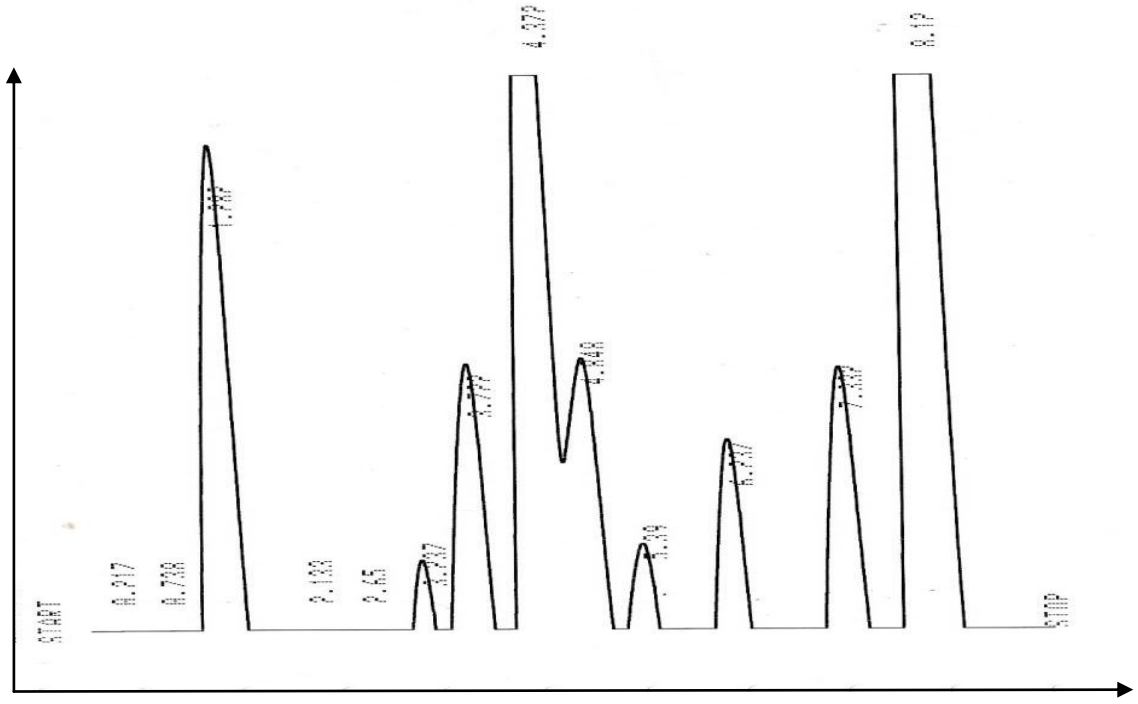

Fig. 1: HPLC Chromatography Of Crude Extract Of *Cutandia Memphetica* Showing (8) Peaks Of Phenolic Compounds

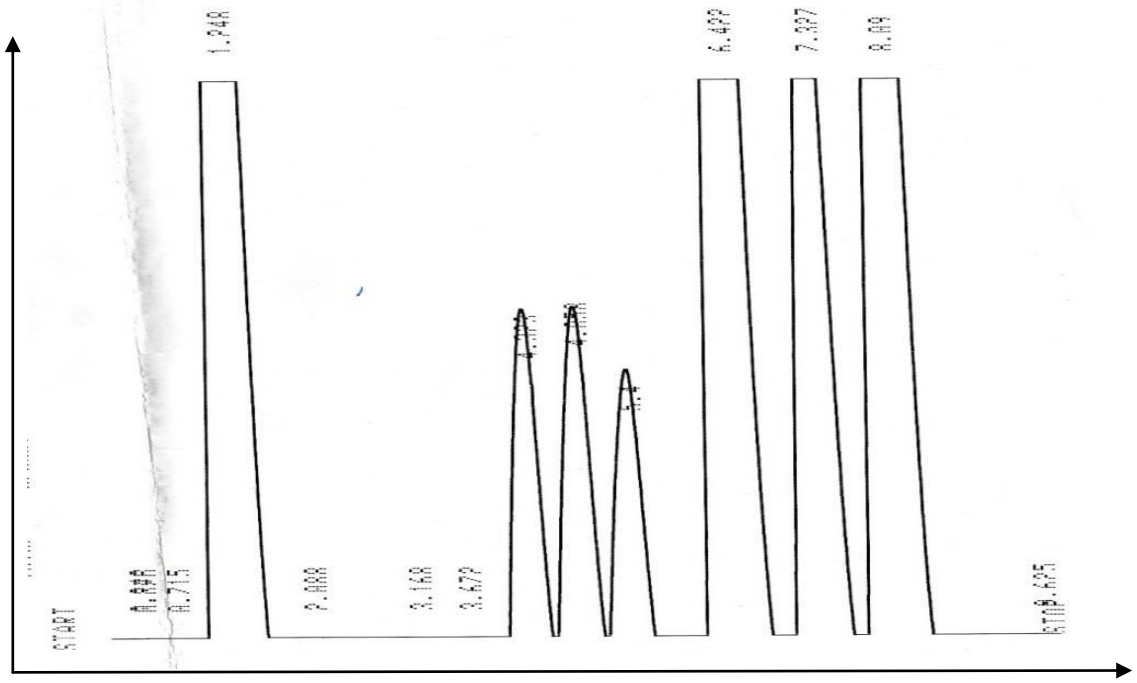

Fig. 2: HPLC Chromatography Of Crude Extract Of *Cutandia Dichotoma* Showing (7) Peaks Of Phenolic Compounds

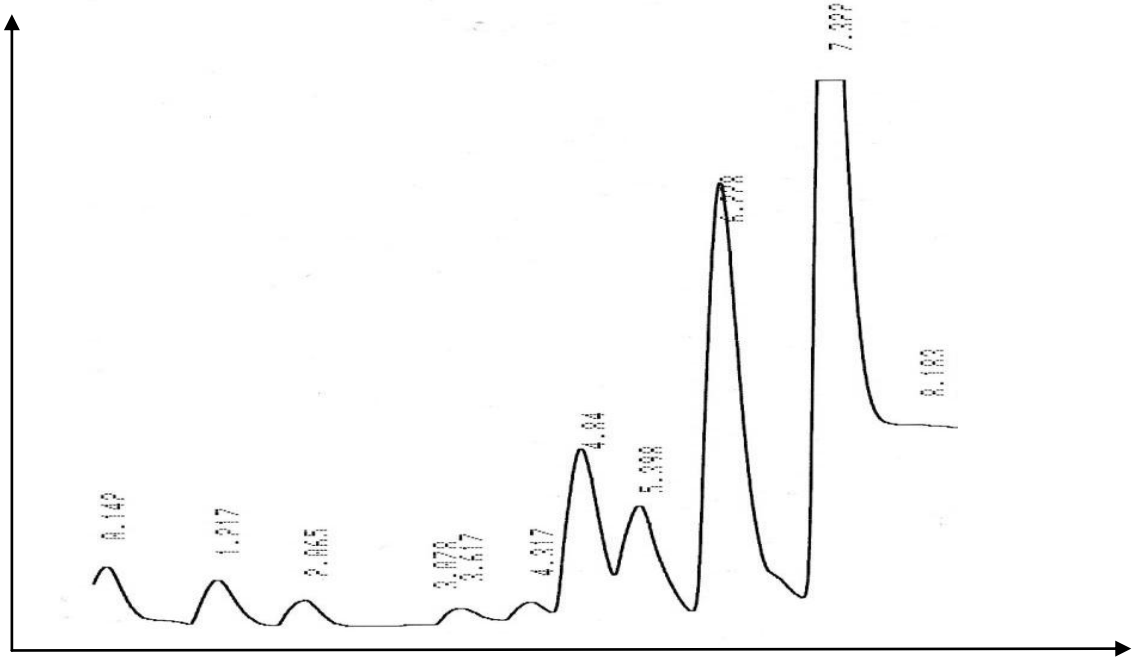

Fig. 3: HPLC Chromatography Of Crude Extract Of *Dactylis Glomerata* Showing (8) Peaks of Phenolic Compounds

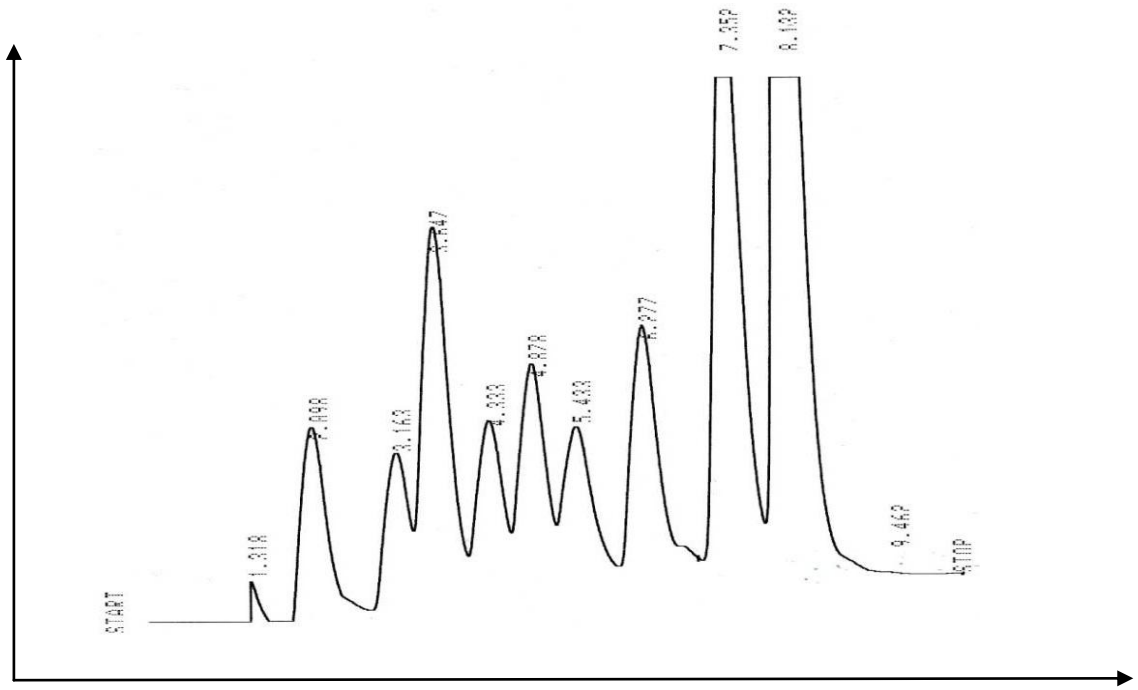

Fig. 4: HPLC Chromatography Of Crude Extract Of *Echinaria Capitata* Showing (8) Peaks Of Phenolic Compounds

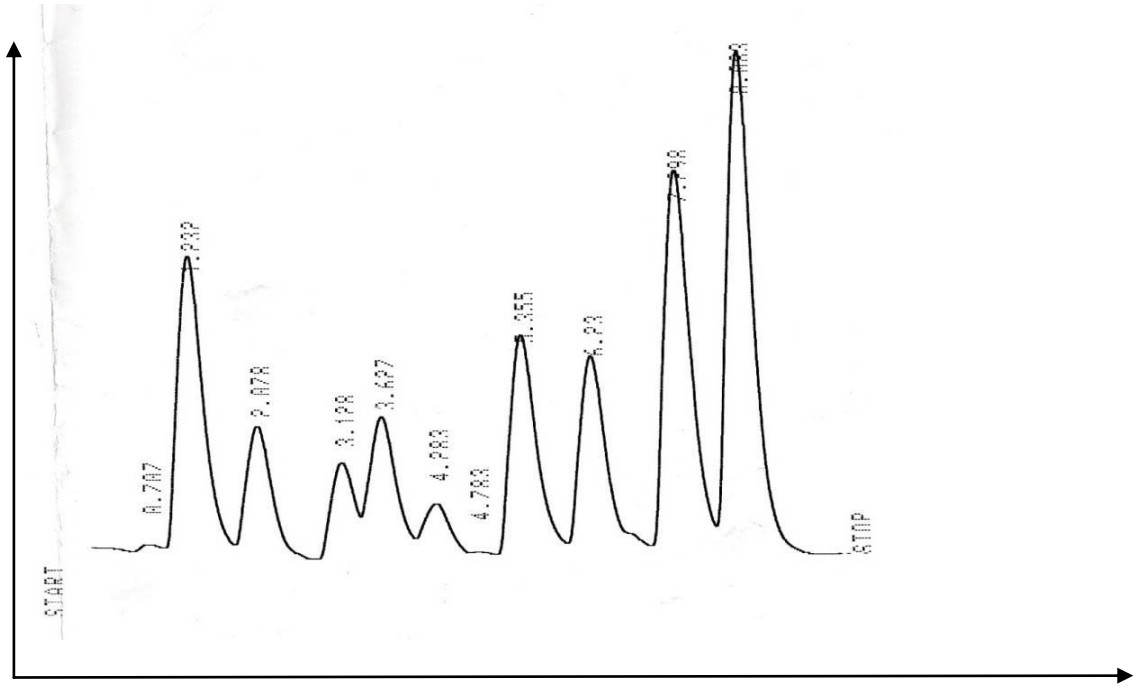

Fig. 5: HPLC Chromatography Of Crude Extract Of *Poa Annua* Showing (8) Peaks Of Phenolic Compounds

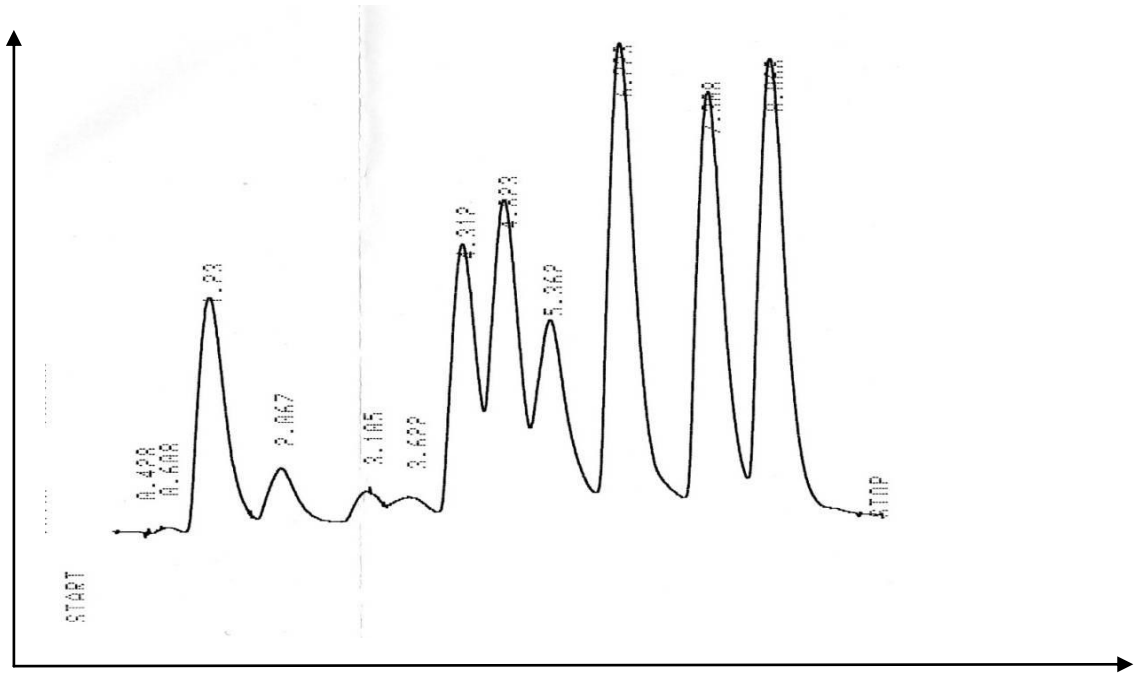

Fig. 6: HPLC Chromatography Of Crude Extract Of *Poa Angustifolia* Showing (8) Peaks Of Phenolic Compounds

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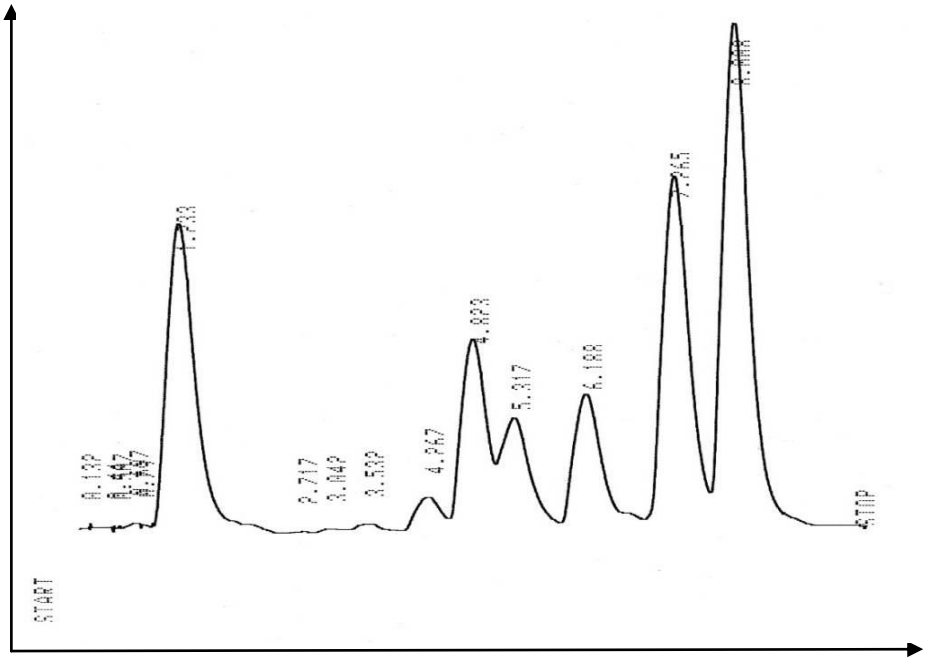

Fig (7) : HPLC chromatography of crude extract of *Poa pratensis* showing (6) peaks of phenolic compounds

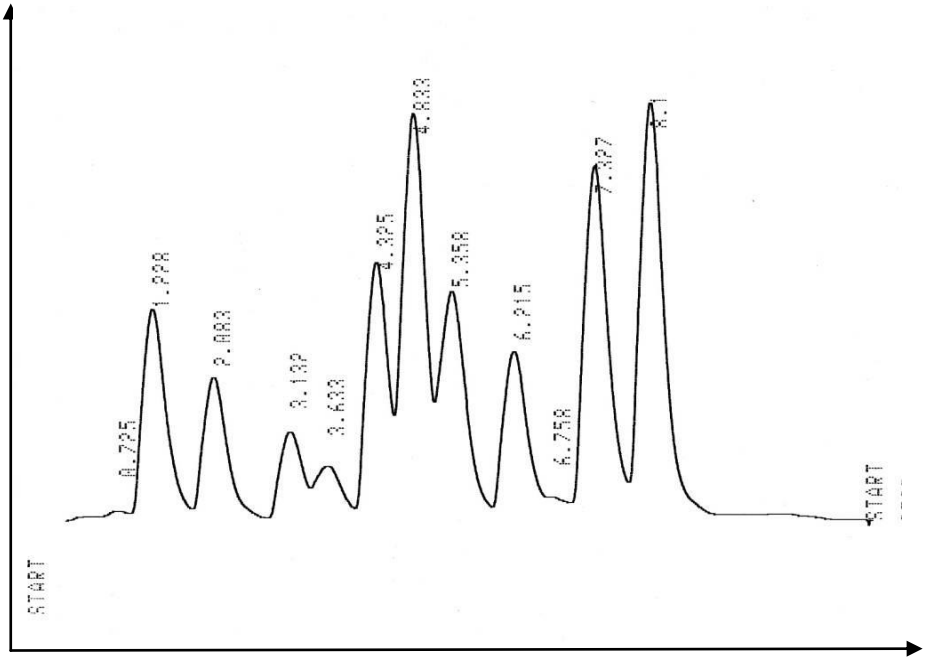

Fig (8) : HPLC chromatography of crude extract of *Poa triviales* showing (8) peaks of phenolic compounds

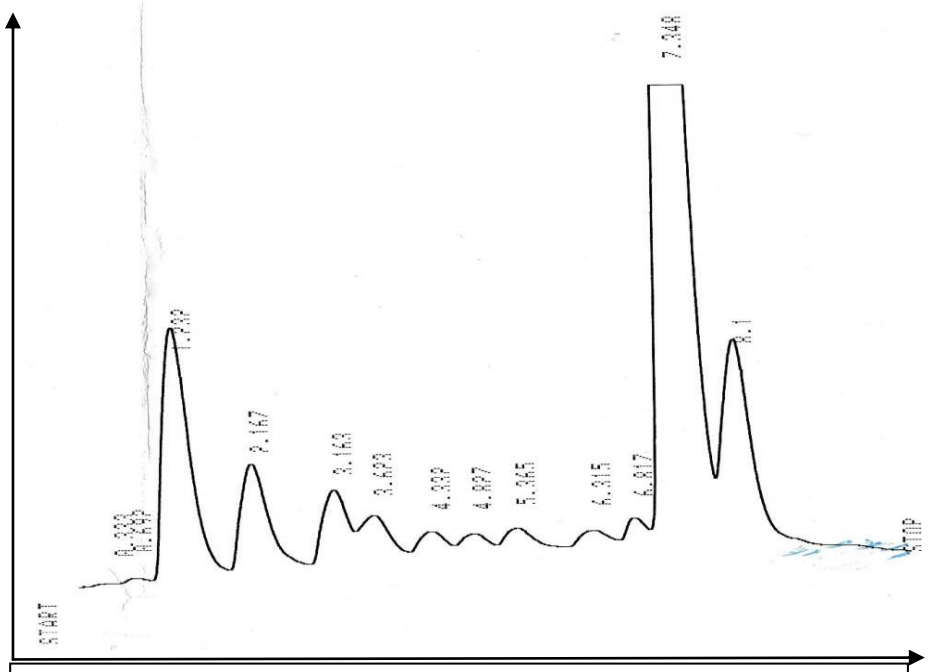

Fig (9): HPLC chromatography of crude extract of *Psilurus incurvus* showing (8) peaks of phenolic compounds

Table 1: Phenolic Compounds Number With Its Standard Space Pack Measurements

P. C. No.	Phenolic compounds	Space pack measurement
1.	Chlorogenic acid	٨٣٠١
2.	Homoorientin	١٠١٥٨
3.	Myriscetin	١٣٨٧٠
4.	Kaempferol	١٧٧٧٨
5.	Anthraquinon	١٢٤٦٠
6.	Rutin	١٤٤٧١
7.	Quercetin	١٦١٠٧
8.	Luteolin	١٥٨٩٤

Table 2: Space Pack Materials of Phenolic Compounds in Some Species of Some Genera of The Tribe (*Poeae*) in Iraq

Species	1	2	3	4	5	6	7	8
Cutandia dichotoma	147553	17683	10848	45138	48049	-	91920	151558
Cutandia memphetica	70135	15073	27414	95783	36615	43974	48400	136585
Dactylis glomerata	3382	1954	1090	1163	9180	34959	68358	11057
Eremopoa persica	-	-	-	-	-	-	-	-
Echinaria capitata	18395	46282	27210	28068	31137	42080	68509	114305
Poa angustifolia	17893	5911	3064	19554	17632	37682	32129	34523
Poa annua	21913	9588	6382	4228	16996	16235	29253	37649
Poa pratensis	28206	-	-	2681	10249	12349	30151	43004
Poa triviales	13982	9503	5453	15759	16327	11445	24568	28866
Psilurus incurvus	23496	11626	7495	3850	4325	3851	112690	21110

Table 3: Total Phenolic Compounds in Some Species of Some Genera of The Tribe (Poeae) in Iraq

Species	1	2	3	4	5	6	7	8
Cutandia dichotoma	p	p	p	p	p	a	p	p
Cutandia memphetica	p	p	p	p	p	p	p	p
Dactylis glomerata	p	p	p	p	p	p	p	p
Eremopoa persica	a	a	a	a	a	a	a	a
Echinaria capitata	p	p	p	p	p	p	p	p
Poa angustifolia	p	p	p	p	p	p	p	p
Poa annua	p	p	p	p	p	p	p	p
Poa pratensis	p	a	a	p	p	p	p	p
Poa triviales	p	p	p	p	p	p	p	p
Psilurus incurvus	p	p	p	p	p	p	p	p

a = absent. P = present

Table 4: Concentrations of Phenolic Compounds (mg/gm) in Some Species of Some Genera of The Tribe (Poeae) in Iraq

Species	1	2	3	4	5	6	7	8
Cutandia dichotoma	2.221.91	217.59	97.76	317.37	482.03	-	713.35	1.191.91
Cutandia memphetica	1.056.12	185.48	247.06	673.47	367.32	379.85	375.61	1.074.18
Dactylis glomerata	50.93	24.05	9.82	8.18	92.09	301.97	530.49	86.96
Eremopoa persica	-	-	-	-	-	-	-	-
Echinaria capitata	276.99	569.52	245.22	197.35	312.37	363.49	531.67	898.96
Poa angustifolia	269.44	72.74	27.61	137.48	176.88	325.49	249.34	271.51
Poa annua	329.91	117.98	57.51	290.72	170.51	140.23	277.02	296.08
Poa pratensis	424.74	-	-	18.85	102.82	106.67	233.98	338.21
Poa triviales	210.54	116.94	49.14	110.81	163.79	98.86	190.66	227.02
Psilurus incurvus	353.81	143.06	67.55	27.07	43.39	33.26	874.54	166.02

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