Chemotaxonomical study of the genera *Brunnera* (Schenk) Jonston,*Choriantha* H.Rirdel., *Cynoglossum* Mill., *Solenanthus* Ledeb. & *Symphytum* (Boiss.) L. (Boraginaceae) in Kurdistan region of Iraq by using High Performance Liquid Chromatography (HPLC).

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Abstract

In the present study, seven of the phenolic compounds, in seven plant species within five genera of the Boraginaceae family have been identified which were (*Brunnera orientalis* (Schenk) Jonston., *Choriantha popoviana* H., *Cynoglossum creticum* Mill, *Solenanthus circinnatus* Ledeb, *Solenanthus stamineus* Defed, *Symphytum kurdicum* Boiss and *Symphytum tuberosum* L.). were studied in order to important the phenolic profile. The chemical composition of these species were examined for the content of the following phenolic compounds:Caffeic acid , Estragole,2-6Dimethyl phenol, Coumaric acid, Eugenol,Salicylic acid, and P-Cresol, by using high performance liquid chromatography (HPLC).The results showed that the most abundant phenolic acids were: Coumaric acid and Salicylic acid which were found in all the studied taxa, followed by Caffeic acid was absent from *Brunnera orientalis* and *Solenanthus circinnatus* while Eugenol was absent just from *Symphytum kurdicum* Boiss and *Symphytum tuberosum* L. , also 2-6Dimethyl phenol was absent from *Choriantha popoviana* and *Cynoglossum creticum*, whereas the less prevalent phenolic compounds were Estragole(4- Allyl anisole) and P-Cresol which found in just two of the

studied taxa (the first found in *Solenanthus stamineus* and *Symphytum tuberosum* L. while the second one found in *Choriantha popoviana* H. and *Symphytum kurdicum* Boiss.). The different distribution for the presence of phenolic compounds in different species was of benefit taxonomic value and can be used to enhance taxonomic studies to isolate and identify plant and do not less important as other taxonomic studies the present study regards as the first study of these Boraginaceae family genera in Iraq..

Key words: Chemotaxonomical study, Boraginaceae, High performance liquid chromatography (HPLC), **Kurdistan region of Iraq**.

دراسه تصنيفيه كيميائيه للاجناس ***Brunnera*** **(Schenk) Jonston,*Choriantha* H.Rirdel., العائده للعائله *Cynoglossum* Mill., *Solenanthus* Ledeb. & *Symphytum* (Boiss.) L. (Boraginaceae) في اقليم كوردستان العراق باستخدام تقنية HPLC .**

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الخلاصة

في الدراسة الحالية تم تشخيص سبع مركبات فينولية في سبع انواع من النباتات ضمن خمس اجناس من العائلة Boraginaceae وهي *Brunnera orientalis* (Schenk) Jonston. و *Choriantha popoviana* H. Rirdel و *Cynoglossum creticum* Mill و *Solenanthus circinnatus* Ledeb و *Solenanthus stamineus* Defed و *Symphytum kurdicum* Boiss و *Symphytum tuberosum* L. تمت دراستها من خلال تشخيص المركبات الفينولية. تم فحص التركيب الكيميائي لهذه الأنواع عن محتواها من المركبات الفينولية التاليه :- Caffeic acid , Estragole,2-6Dimethyl phenol, Coumaric acid, Eugenol,Salicylic acid and P-Cresol باستخدام تقنية.high performance Liquid chromatography (HPLC) وأظهرت النتائج مايلي:- من المركبات الفينوليه الاكثر وفرة مابين الانواع المدروسه هما Coumaric acid و Salicylic acid اللذين عثر عليهما في جميع الأنواع المدروسة، يليهما Caffeic acid والذي وجد في جميع الانواع ماعدا النوعين *Brunnera orientalis* و *Solenanthus circinnatus* اما Eugenol فوجد في جميع الانواع ماعدا انواع الجنس *Symphytum* كذلك 2-

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6Dimethyl phenol وجد في جميع الانواع عدا النوعين *Choriantha popoviana* و *Cynoglossum creticum* ، في حين أن المركبين الأقل انتشارا هما Estragole(4- Allyl anisole) و P-Cresol اذ وجد كل منهما في اثنين فقط من الانواع المدروسة (الاول وجد في *Symphytum tuberosum* , *Solenanthus stamineus*. اما الثاني فقدوجد في *Symphytum kurdicum* , *Choriantha popoviana*). لذا فأن التوزيع المختلف لتواجد المركبات الفينولية باختلاف الانواع يعتبر ذو فائدة تصنيفية مهمه يمكن استخدامها لتعزيز الدراسات التصنيفية في عزل وتشخيص النباتات ولاتقل اهميه عن الدراسات التصنيفيه الاخرى وتعتبر هذه الدراسة هي الاولى التي تم اجرائها على نباتات الاجناس قيد الدراسة في العراق .

الكلمات المفتاحية: Boraginaceae، Chemotaxonomical study، تقنية (HPLC)، إقليم كوردستان العراق.

Introduction

Since the early 1960s, phytochemical characters started to attract the attention of plant taxonomists and rapidly expanding areas of plant taxonomy and how to use the chemical information to improve the classification of the plant. In fact chemotaxonomy has various ancient origins, perhaps foremost come the search by herbalists and pharmacologists, for drugs, that have involved the accumulation of information on the chemical content of a very wide range of plants, second major ancient origin of chemotaxonomy were the field of morphology and anatomy, for example, color, crystals and starch which differ in morphology and chemical composition, (Stace ,1980), also based on (Stace ,1980).The phenolic compounds which dissolved in the water are the first groups of chemical compounds used in chemical classification, (Smith, 1976). It is known that the taste and smell of plants or both play an important role in distinguishing some overlapping taxa, including species and varieties, regardless of any other description (Al-Musawi,1987;Al-Mashhadany,1992). The phenolic compounds are used by a large number of researchers to solve taxonomic problems (BateSmith, 1948,1958; Harborne, 1964,1967a, 1967b; Ribereau, 1972; Cutler, 1969,1972;Rezende & Gottlieb, 1973; Blatt *et al*., 1994 and Sandor, 1994). Boraginaceae including about 100 genera with 2000 species in all over the world is divided into four subfamilies: Boraginoideae, Heliotropioideae, Cordioideae and Ehretioideae (Gottschling *et*

al., 2001).ButStevens (2001) indicated that the Boraginaceae comprises about 2740 species distributed in 148 genera. Alkaloids, naphthoquinones, polyphenols, phytosterols, terpenoids and fatty acids were the main secondary metabolites of the Boraginaceae(Zhou & Duan, 2005; Iqbal *et al*., 2005). Polyphenols including phenolic acids and flavonoids distributed in the Boraginaceae have diverse pharmaceutical activities such as antioxidant, anti-inflammatory, anti-viral, anti-bacterial and hepato- faprotecting activity (Wu, 1990; Zeng & Zeng, 1994; Iqbal *et al*., 2005). One of the chemical compound groups used in chemotaxonomy is water soluble phenolic compounds of plant samples (Smith, 1976). Phenolic compounds were used by (Bate-Smith,1948 ; Bate-Smith *et al.* 1967; Harborne, 1964, ; Harborne, 1967b; Ribereau-Gayon, 1972; Cutler, 1969; 1972; Rezende & Gottlieb , 1973; Blatt *et al*., 1994 and Sandor , 1994) to solve taxonomical problems. Phenolic compounds consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids (Khoddami *et al*.2013). The use of the distribution patterns of natural plant product-alkaloids,terpens,phenolics,glucosinolites,terpenoids and carbohydrates is well- established as amajor tool for investigating population structures,speices,taxonomical problems and phyletic relationships of genera.Taxonomically ,the most important phenolics are the flavonoids, which have relatively common nucleus with great variety of types and patterns of side-groups that characterize the individual compounds.There is usually a considerable diversity of flavonoids in species (Nakipoklu,2002). The present study aimed to study the phenolic compounds in some genera within Boraginaceae family that have not been studied before. The species within the studied genera were *Brunnera orientali* , *Choriantha popoviana* , *Cynoglossum creticum* , *Solenanthus circinnatus* , *Solenanthus stamineus* , *Symphytum kurdicum* and *Symphytum tuberosum* that some of them found at high altitudes reaches to 2700 m.The identification process of the phenolic compound has been done by using high performance Liquid chromatography (HPLC).

Materials and Methods

Sample collection and Preparation:

Samples have been were obtained from deferent locations in Kurdistan region of Iraq in April to september (2014 and 2015). The Harborne method was followed for extraction of phenolic compound in vegetative plant parts as follows, (Harborne1973) .

Leaves and stems of the samples were dried at 25 °C in darkness and analyzed after grinding in a household blender. All samples were analyzed within 3 months of collection.The extraction method used for dried samples as follows: 50 ml of%70 methyl alcohol was added to 5 gm of dried sample, and left at room temperature for 48 hrs. The extraction mixture then filtered, and then the extraction was concentrated to adequate volume in order to get rid of alcohol by using air conditioner in as much as volume of Petroleum Ether (80-100 boiling point) was added to the product, mixture shacked gently, placed in separating funnel and left for some time to separated clearly into two layer. There by the major part of chlorophyll dissolved in petroleum ether, and float because of its lesser density than water extraction of phenolic compounds that dissolve in water and make the lower layer, which drown from lower of funnel and injected to HPLC .

HPLC Analysis

The analytical HPLC system employed consisted of high performance liquid chromatograph apparatus in the Sulaimani Polytechnic University, Agricaltural Technical College. The separation was achieved on Analytical column: Eurospher 100, C18, 5µm, 250 x 4.6 mm at ambient temperature. The mobile phase consisted of water-acetonitrile water: concentrated phosphoric acid (400:600: 3± 0.05). The flow rate was 0.8 mL/min and the injection volume was 20 µL. The monitoring wavelength was 254. The identification of each compound was based on a combination of retention time and spectral matching.

Results and Discussion

This research was done in pure standards Conditions that used with HPLC method. The present study included delimitation of a quality of phenolic compounds in the studied species,

depending on some available standard materials. The kinds of phenolic compounds which detected in the samples are presented in Table (1) with the Retention times of each of them and the standard curves of them illustrated in (Figure 2) with their structure (figure 1) .

Table (1) Retention time of standard phenolic compounds by (HPLC).

No.	Compound names	Retention time (minute)
1	Caffeic acid	3.431
2	Coumaric acid	3.796
3	Eugenol	4.401
4	Estragole(4- Allyl anisole)	5.751
5	2-6Dimethyl phenol	5.936
6	Salicylic acid	5. 951
7	P-Cresol	7.855

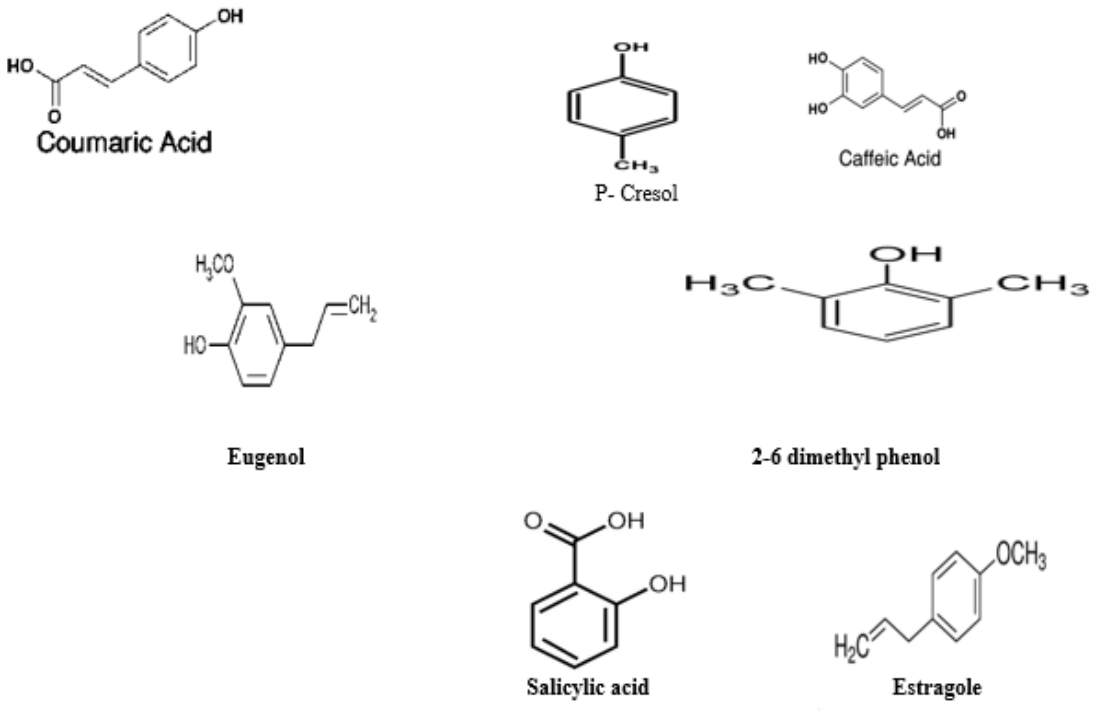

Fig. (1) Chemical structures of standards phenolic compounds. (Dewick, 1997).

The results in Table (2) and figure (2(A,B) ,3,4,5,6,7,8,9) shows the kinds of phenolic compounds which obtained by methanolic extracts of the plant material from the seven samples. According to available standards compounds the results were as follows (Table1, 2):

1-Coumaric acid and Salicylic acid present in all studied taxa.

2- Caffeic acid was revealed in species Choriantha popoviana, Cynoglossum creticum , Solenanthus stamineus , Symphytum kurdicum , Symphytum tuberosum .

3- Eugenol was found in species Brunnera orientalis , Choriantha popoviana , Cynoglossum creticum , Solenanthus circinnatus and Solenanthus stamineus .

4- 2-6Dimethyl phenol was existing in species Brunnera orientalis , Solenanthus circinnatus and Solenanthus stamineus .

5- Estragole(4- Allyl anisole) was identified in two species, *Solenanthus stamineus* and *Symphytum tuberosum* .

6- P-Cresol was found in two species also , *Choriantha popoviana* and *Symphytum kurdicum* . But according to numbers of phenolic compounds, the studied species might divides into three parts Table (1,2):

a- Species contain six phenolic compounds such as *Solenanthus stamineus* (1, 2, 3, 4, 5,6).

b- Species contain five phenolic compounds such as *Choriantha popoviana* (1, 2, 3, 6, 7). C- Species contain four phenolic compounds such as *Brunnera orientalis .and Solenanthus circinnatus* (2, 3, 5, 6) , *Cynoglossum creticum* (1, 2, 3, 6) , *Symphytum kurdicum* (1, 2, 6, 7) and *Symphytum tuberosum* (1, 2, 4, 6).

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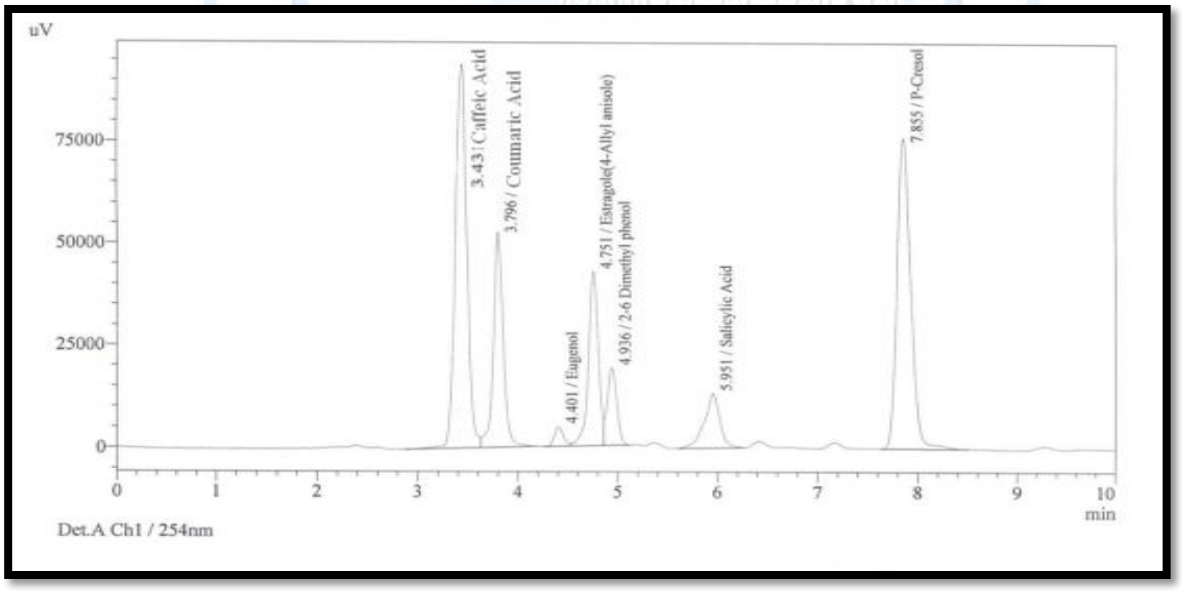
Table (2) Distribution of phenolic compounds in species.

Species	Phenolic compounds						
	Caffeic acid	Coumaric acid	Eugenol	Estragole(4-Allyl anisole)	2-6Dimethyl phenol	Salicylic acid	P-Cresol
Brunnera orientalis		X	X		X	X	
Choriantha popoviana	X	X	X			X	X
Cynoglossum creticum	X	X	X			X	
Solenanthus circinnatus		X	X		X	X	
Solenanthus stamineus	X	X	X	X	X	X	
Symphytum kurdicum	X	X				X	X
Symphytum tuberosum	X	X		X		X	
Number of taxa	5	7	5	2	3	7	2



A

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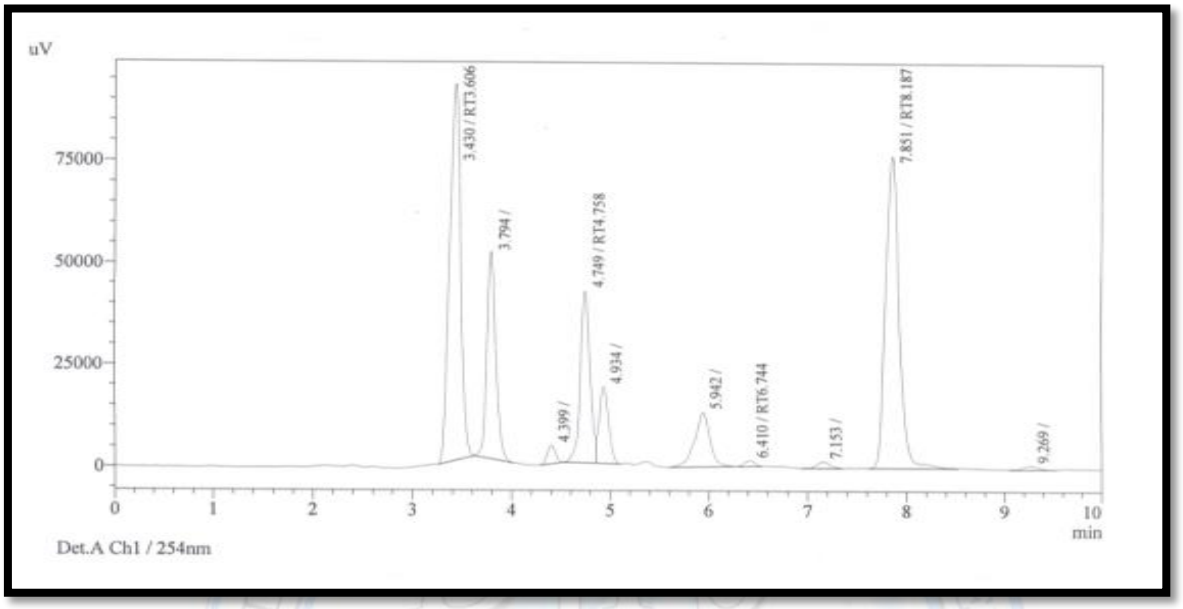

B

Fig. 2(A& B) Diagram of standards phenolic compounds by (HPLC).

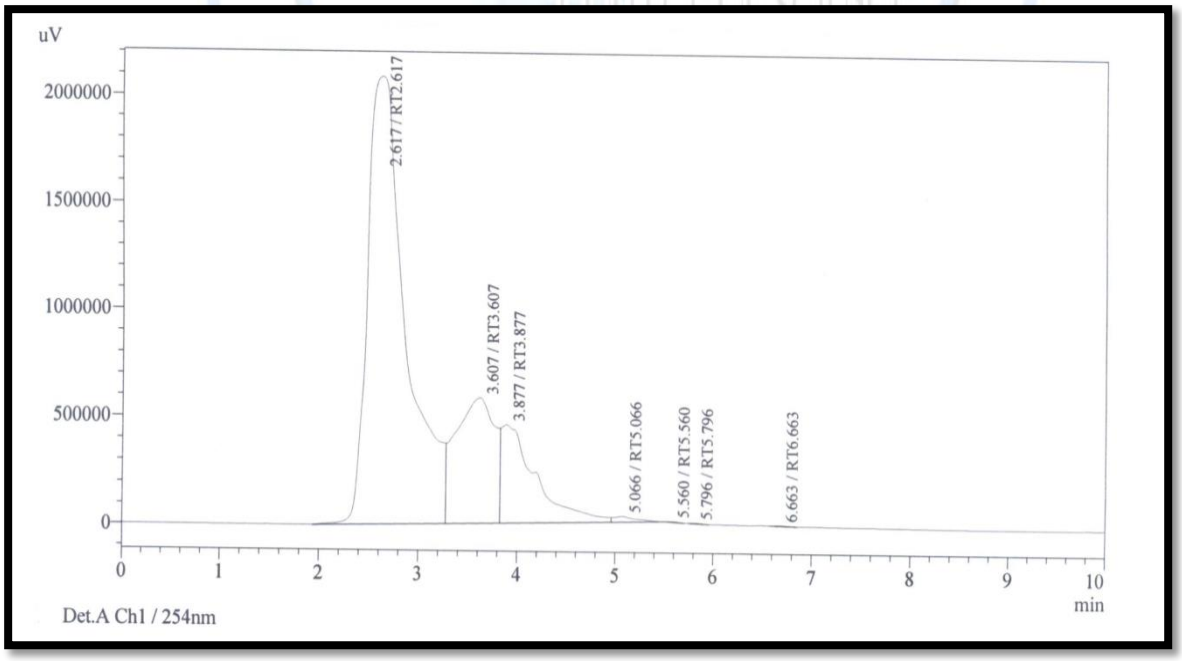

Fig. (3) Typical HPLC chromatograph of *Brunnera orientalis*.

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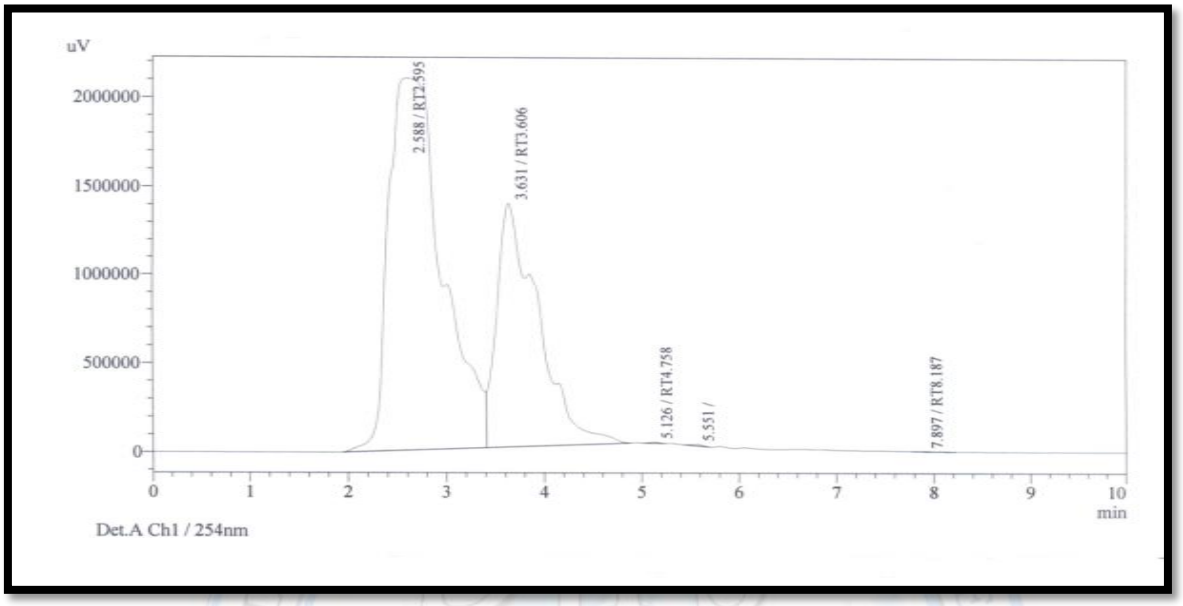

Fig. (4) Typical HPLC chromatograph of *Choriantha popoviana*.

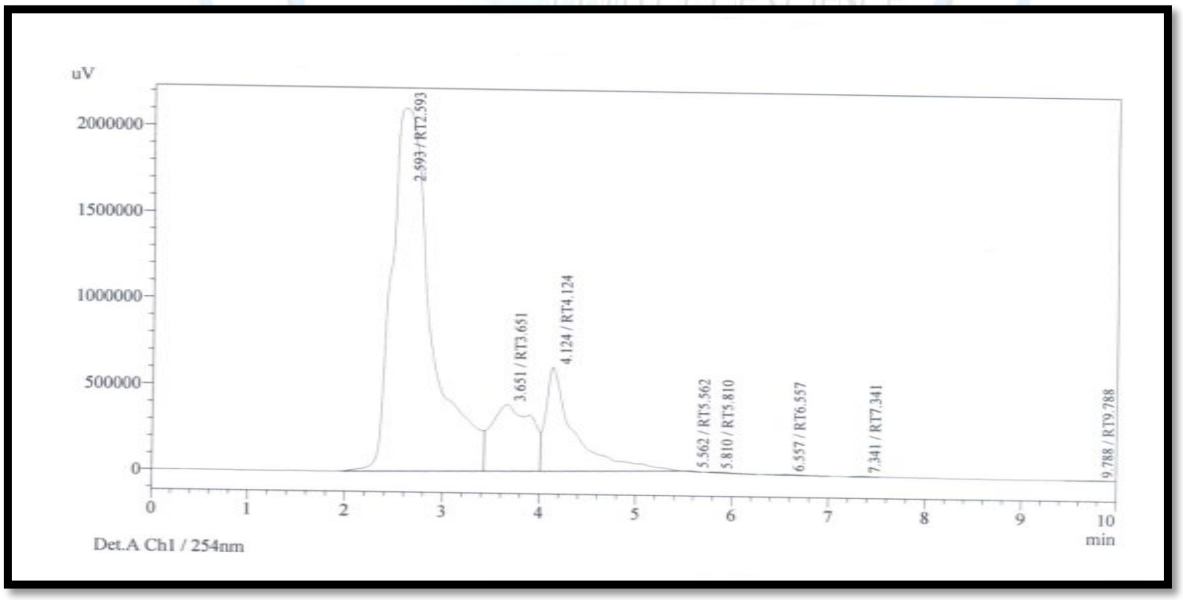

Fig. (5) Typical HPLC chromatograph of *Cynoglossum creticum* .

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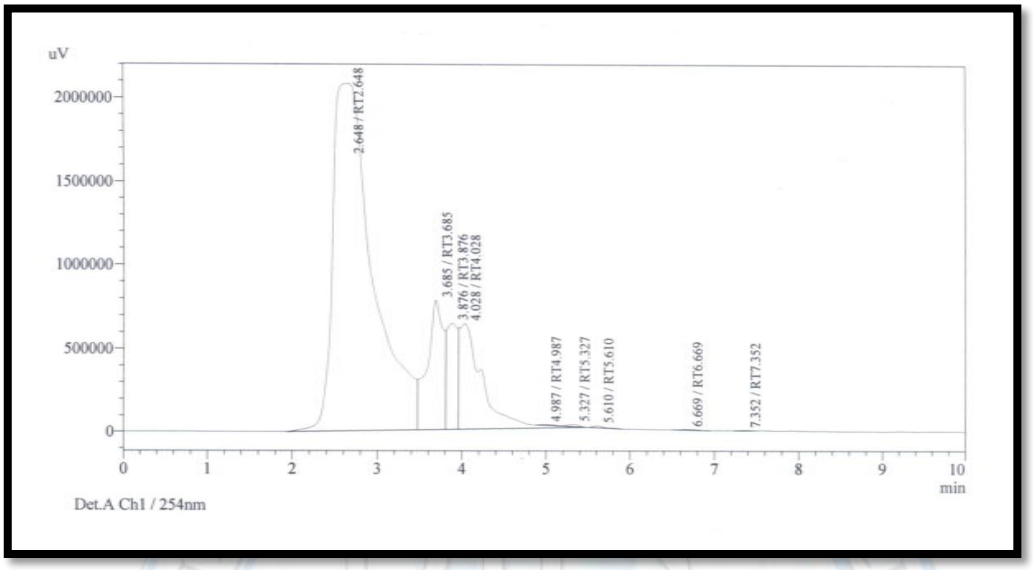

Fig. (6) Typical HPLC chromatograph of *Solenanthus circinnatus* .

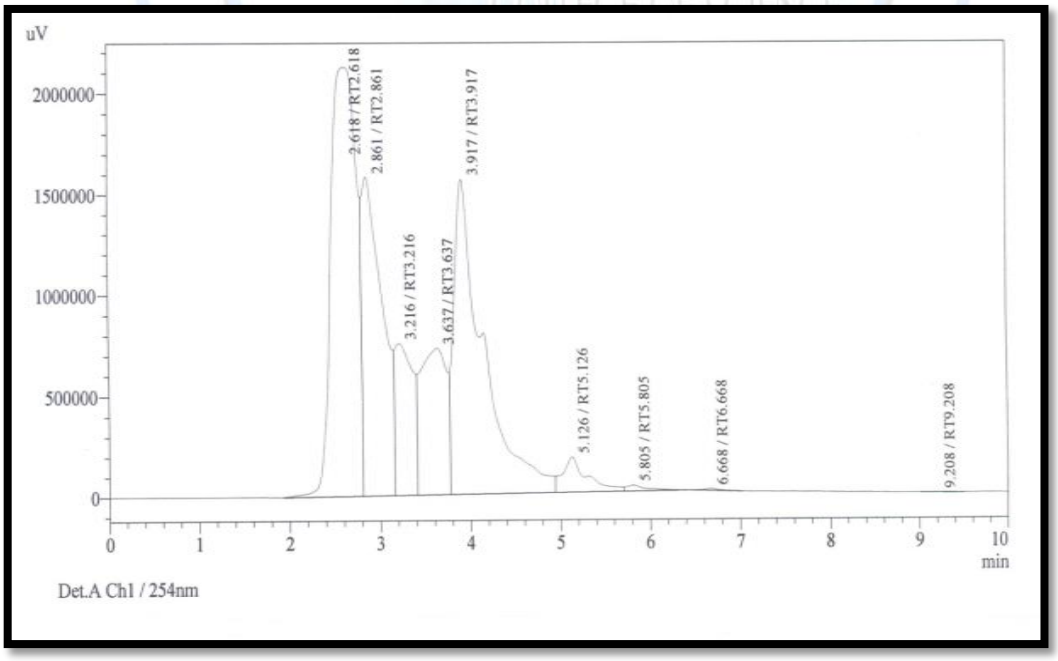

Fig. (7) Typical HPLC chromatograph of *Solenanthus stamineus* .

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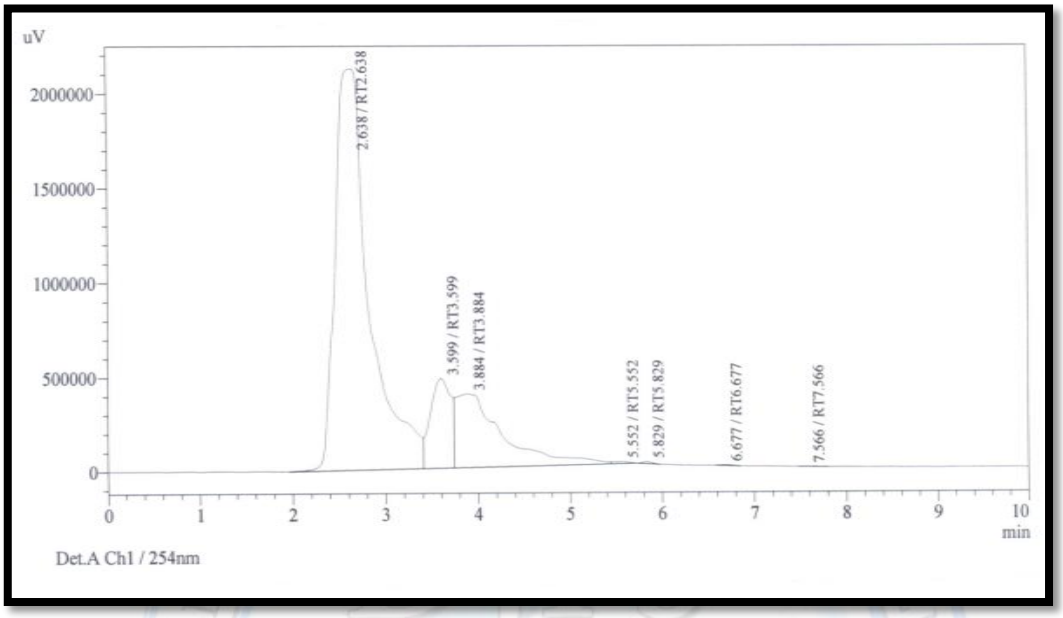

Fig. (8) Typical HPLC chromatograph of *Symphytum kurdicum* .

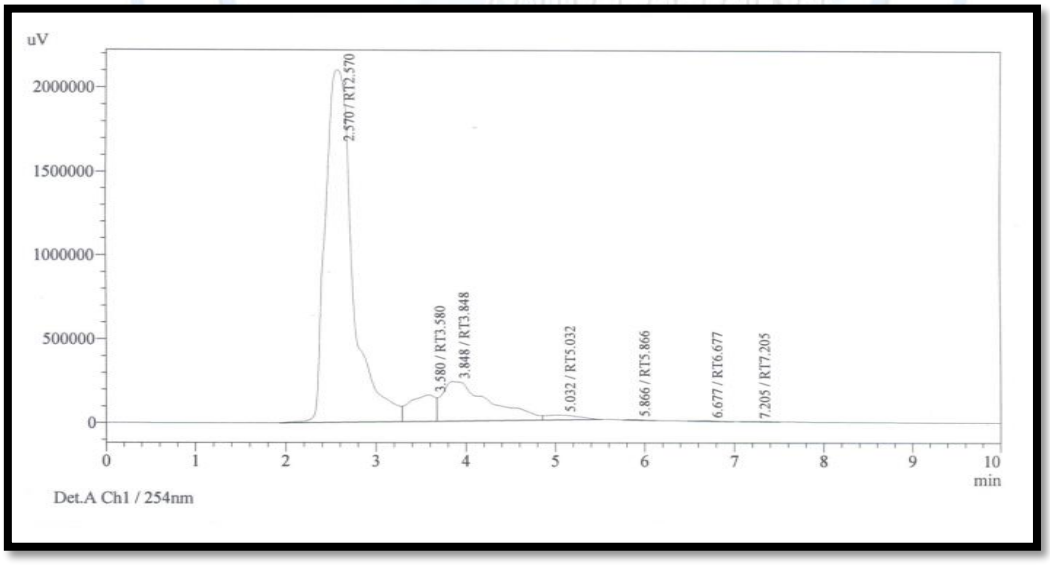

Fig. (9) Typical HPLC chromatograph of *Symphytum tuberosum*.

So there were contrasts in the phenolic profile of the contemplated species due to the reverence in their genomic structure and this is in compatible with (Proestos and Komaitis, 2013) who found that the nearness of polyphenols in any plant is generally affected by hereditary variables .

Varying interpretation and evaluation of morphological characters very often result in disagreement regarding classification. In such instances taxonomists as a rule look for characters other than morphological ones (Erdtman 1952; Wodehous1959 ; Benson1962 ; Davis & Heywood1963) characters are considered first. Sometimes they produce convincing evidence and sometimes they fail to do so. In such situations, chemical characters may become very useful guides to taxonomists. At present, one important task of chemotaxonomy consists in procuring additional evidence in all cases of obscure relationships of plants.

Therefore the determination of phenolic content in any plant by using HPLC technique is very important in solving many of the taxonomic problems as well as It extremely reduces time and efforts compared with other chromatographic method.

Conclusions

To use phenolic compounds more widely as genetic markers, these would have to be not only universal and abundant but also environmentally stable and convenient for identifying taxonomic position (Fairbbothers *et al*.,1975). By reviewing the resources available, it was clear that the current study is the first to address the genera of races above developing in Iraq has been the current study dealt with determining the quality of phenolic compounds in taxa races the above as the method is used high performance Liquid chromatography (HPLC). As mentioned earlier, and with the help of standard phenoles that we were able to be provided with the adoption of a seven standard phenoles Figure 1 and Table 1. Expressed taxa races the above important variations in the content of phenolic compounds and build on the results of these compounds can adopt taxonomic evidence of no less importance than the other phenotypic traits, including the anatomical and chromosomal, environmental and pollen.

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