ANALYSIS OF NATURALLY OCCURRING PENOLIC COMPOUNDS OF THE GENERA *CLINOPODIUM* L. , *HYMENOCRATER* FISH & C. A. MEY. AND *MELISSA* L.

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ABSTRACT

Six taxa of Lamiaceae family including three taxa of the genus *Clinopodium* L. *(C. vulgare* subsp. v*ulgare*L., *C. umbrosum* (M. B.) C. Koch. and *C. congstum* Boiss. & Hausskn ex. Boiss.) and two of the genus *Hymenocrater* Fish. & C. A. Mey. *(H,longiflora* Benth. *H. bituminosus* Fish & C. A. Mey) and one of the genus *Melissa (M.officinalis* subsp.*fficinalis* L.) were studied in order to evaluate their phenolic profile ,as these taxa were studied in such detail for the first time in Kurdistan region of Iraq . The examined phenolic substances which studied in these taxa include: Caffeine, Coumarin, Eugenol Estragole(4- Allyl anisole), 2-6 Dimethyl phenol, Salicylic acid and P-Cresol, by using high performance liquid chromatography (HPLC) technique. The results showed that the most abundant phenolic compound were: Caffeine and 2-6Dimethyl phenol which found in all the studied taxa, followed by Eugenol and Salicylic acid which found in five of the six studied taxa, whereas the less prevalent phenolic compounds were (Coumarin and Estragole(4- Allyl anisole),which found in just two of the studied taxa. The deference in distribution of the phenolic compound in the studied taxa can be utilized to differentiate between them in studies of genetic diversity and chemotaxonomy as it can be possible to distinguish between the genera *Clinopodium*, *Hymenocrater* and *Melissa* beside the distinguish between the taxa of the same genus (*Clinopodium*), using phenolic profile.

Key words: taxa, Lamiaceae, High performance liquid chromatography (HPLC).

تحليل المركبات الفينولية الطبيعية الموجودة في بعض اجناس ال *Clinopodium* ، *Hymenocrater* و *Meliss*

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المستخلص

تم دراسة ستة مراتب تصنيفيه من العائلة الشفوية بينهم ثلاث من الجنس *Clinopodium* هي: (*C. umbrosum*, C. *vulgare* subsp *vulgare*) و *C. congstum*. واثنان من الجنس *Hymenocrater* هما *H. longiflora*. *H. bituminosus* وواحد من الجنس *Melissa* هو (*M. officinalis* subsp. *officinalis* L.) وذلك بالكشف عن نوع المركبات الفينوليه فيها باستخدام تقانة الكروماتوغرافيا السائلة عالية الاداء(HPLC). و تعد هذه الدراسة هي الاولى من نوعها للمراتب التصنيفيه اعلاه في إقليم كردستان العراق. اما الفينولات التي تم الكشف عنها فهي: 2-6Dimethyl phenol, Caffeine Coumarin, Eugenol Estragole(4- Allyl anisole),Salicylic acid و P–Cresol أظهرت نتائج هذه الدراسه أن المركبين الفينوليين الأكثر وفرة هما: Caffeine و2-6Dimethyl phenol اللذان تواجدا في جميع المراتب التصنيفيه اعلاه، يليهما Eugenol وSalicylic acid فاظهرا تواجدهما في خمس من المراتب الستة المدروسه ، في حين كان المركبان الفينوليان الأقل انتشارا هما Coumarin و(Estragole(4- Allyl anisole واللذان وجدا في مرتبتين تصنيفيتين من بين المراتب التصنيفيه المدروسه. ان اختلاف توزيع المركبات الفينوليه في المراتب التصنيفيه المدروسه يمكن عدها كدلائل تصنيفية مهمة تدعم الدراسات الاخرى كالتغايرات الوراثيه والتصنيف الكيميائي ولذا امكن استخدام نتائج هذه الدراسه للتمييز مابين الاجناس *Clinopodium* , *Hymenocrater* and *Melissa* اضافه الى امكانية استخدامها للتمييز مابين المراتب التصنيفيه العائده للجنس *Clinopodium* اعتمادا على نوع المركبات الفينوليه التي تحتويه.

كلمات مفتاحية: مراتب تصنيفية، العائلة الشفوية، تقانة الكروماتوغرافيا السائلة عالية الاداء (HPLC).

INTRODUCTION

The mint family (Lamiaceae) is the sixth largest family of flowering plants in the world and economically important ,with about seven subfamilies, 258 genera (1). Some species are traditionally used as medicinal plants, herbal teas and as raw material in cosmetic Industry. The *Lamiaceae* family is a rich source of polyphenolic compounds (2) however The level of Phenolic compounds are vary greatly within species which can be utilized as comparative data for understanding relationships, and one of main tools of chemotaxonomy (3). Which is one of the more modern and rapidly expanding areas of plant taxonomy as the taste and smell of plants belonging to chemical continents of plant mostly play important role in distinguishing some species, (4). Phenols are a class of chemical compound consisting of OH (hydroxyl) group bonded directly to anaromatic hydrocarbon group.They are classified as simple phenols or polyphenols based on the number of phenol units in the molecule(5) they are also classified by Jeffrey Harborne and Simmonds based on the number of carbons(6). Phenolic compound considered as secondary metabolite in the plants, these are less distributed from the primary metabolite, but with higher taxonomical value. A wide range of phenolic compounds have been reported from the members of this family (7). Besides, interest in phenols has increased greatly because of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential effects on human health (8). In plant materials it was difficult for detection of the phenols, however a number of methods have been proposed for the separation and determination of phenolic compounds mainly based on a high performance liquid chromatography(HPLC) technique (9and 10) and recently modern method of HPLC was conducted for analysis of naturally occurring phenolic compounds in aromatic plants such as the study of Proestos and Komaitis(11) and also applied that on Lamiaceae family such as the study of Verma and Trehan(11) however, because there is no phenolic profile available on the chemical composition of the Lamiaceae species growing in Kurdistan region of Iraq this study was conducted in order to use it for taxonomical relationship between these species**.**

MATERIALS AND METHODS

Sample collection and Preparation

Plant samples of each species were obtained from deferent locations in Kurdistan region of Iraq. 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 had 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 was then filtered, and then the extract was concentrated to adequate volume in order to get rid of alcohol by using air conditioner ,then as much as volume of Petroleum Ether (80-100 boiling point) was added to the product, mixed and shacked gently, placed in separating funnel and left for some time to separated clearly into two layer. Thereby 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 draw from lower of funnel and injected to HPLC apparatus.

HPLC Analysis

All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 62.5% aqueous methanol containing BHT 1 g/L, and 6 M HCL to yield concentrations ranging between 0.5–25 mg/L. Stock working solutions of the standards were stored in darkness at −18 °C (11). The analytical of high performance liquid chromatography apparatus system was employed .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

The kinds of phenolic compounds which detected in the studied species are presented in Table 1 ,with the Retention times of each of them and the standard curves of them using HPLC technique was illustrated in Figure 1,with their structure (figure 2) . The results of the kinds of phenolic compounds which obtained by methanolic extracts of the plant material from the six samples, show that there is a increased variability in the analysed taxa and found that the most abundant phenolic compounds were: Caffeine and2-6Dimethyl phenol which found in all the studied taxa (Table2), followed by Eugenol and Salicylic acid which found in five of the six studied species ,whereas the less abundant phenolic compounds were Coumarin and Estragole which found in just two of the studied taxa.

Table. 1 Kinds of standard phenolic compounds including : Caffeine, Coumarin, Eugenol , Estragole(4- Allyl anisole) ,2-6Dimethyl phenol , Salicylic acid and P-Cresol. with their retention times that used in HPLC technique.

No.	Compound names	Retention time (minute)
1	Caffeine	3.722
2	Coumarin	4.270
3	Eugenol	4.834
4	Estragole(4- Allyl anisole)	5.440
5	2-6Dimethyl phenol	5.676
6	Salicylic acid	6.339
7	P-Cresol	7.625

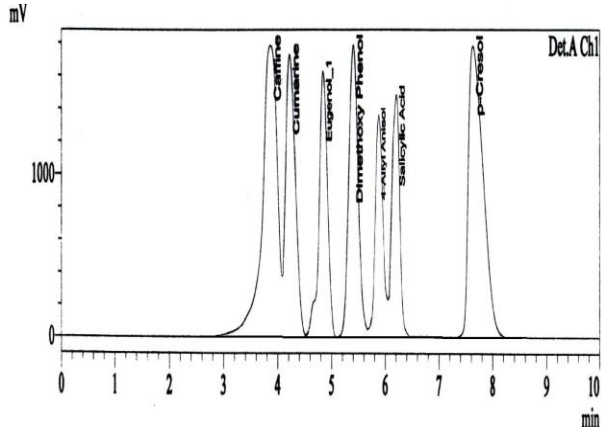

Fig .1 Represent the standard curves of the phenolic substances used in this study including : Caffeine , Coumarin, Eugenol , Estragole(4- Allyl anisole) ,2-6Dimethyl phenol , Salicylic acid and P-Cresol

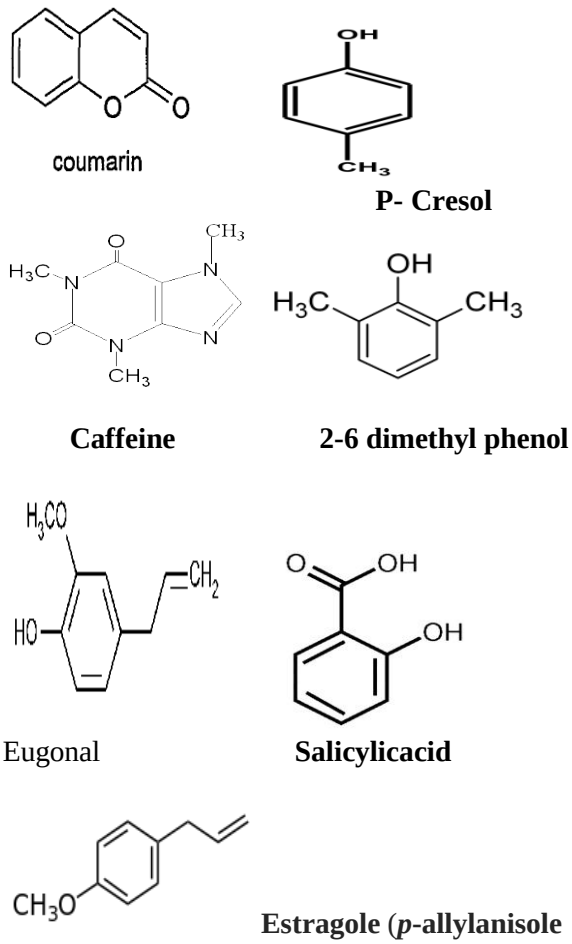

Figure 2 . Structure of the standard phenolic compounds used in this study including Caffeine , Coumarin, Eugenol, Estragole(4- Allyl anisole) ,2-6Dimethyl phenol, ,Salicylic acid and P-Cresol

The identification of each phenolic compound was based on a combination of retention time and spectral matching, since polyphenols absorb in the ultraviolet (UV) region and using aqueous methanol for performing the extraction due Methanol has a protective role. It can prevent phenolic compounds from being oxidized by enzymes, such as phenoloxidases this is in agreement with (13). The deference in distribution of these phenolic compound in the studied species *can* be utilized to differentiate between them in studies of chememotaxonomy. from these results it can be possible to distinguish between the species of the genus *Clinopodium by* their phenolic profile(Figure 3,4,5) , as all the three species of this genus shared the samee phenolic compounds (Caffeine, 2-6Dimethyl phenol, P-Cresol) however *C. congstum* distinguished from the other species by containing another phenolic compound, (Coumarin and Eugenol),

beside *C. umbrosum* species can be distinguish by having the phenolic compound Eugenol in addition to the shared ones, whereas *C. congstum* was the richest of phenolic compounds, it have all the studied phenolic compounds except Coumarin. The two studied species of *the genus Hymenocrater* have its unique profile compared with the other genera as they shared the same phenolic compounds (Caffeine, Coumarin, Eugenol, 2-6Dimethyl phenol, Salicylic acid),(Figure 6,7) and finally the species *Melissa officinalis* was found rich of phenolic compounds, it have all the studied phenolic compounds except Coumarin (figure 8). So there were differences in the phenolic profile of the studied species because of the deference in their genomic structure and this is in agreement with (11) who found that the presence of polyphenols in any plant is largely influenced by genetic factors. At last determination of phenolic content in any plant using HPLC was very necessary as in the event of complex plant matrix selection of appropriate chromatographic condition for HPLC is a matter of great importance as well as a potentials analytical problem and extremely reduce time and efforts compared with other chromatographic method .And this study has it s importance that it was done for the first time on the new conformed taxa in Kurdistan region of Iraq.

Table 2. Kinds of phenolic compounds which detected in every studied taxa as 1 represent Caffeine,2: Coumarin,3: Eugenol,4: stragole(4-Allyl anisole),5: 2-6Dimethyl phenol,6: Salicylic acid and 7: P-Cresol

Species	Phenolic compounds							No, of phenols
	1	2	3	4	5	6	7	
***Clinopodium vulgare* subsp. *vulgare* L.**	X				X	X	X	4
***C. umbrosum* (M. B.) C. Koch.**	X		X		X		X	4
***C. congstum* Boiss. & Hausskn ex. Boiss.**	X		X	X	X	X	X	6
***Hymenocrater longiflorus* Benth.**	X	X	X		X	X		5
***H. bituminosus* Fish & C. A. Mey**	X	X	X		X	X		5
***Melissa officinalis* subsp. *officinalis* L.**	X		X	X	X	X	X	6

Conclusions

The presence of phenolic compounds, usually called polyphenols, in aromatic plants proved by employing high performance liquid chromatography ,which was found in the studied species in deferent profile ,not only between the genera, but also within the species of the same genus ,which add information about the chemical structure of these species and can be utilized for determining taxonomic relationship between the species belong to these genera.

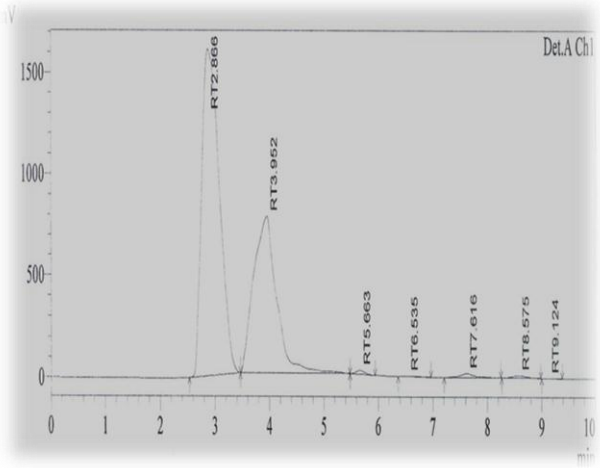

Fig.3 Typical HPLC chromatograph of *the phenolic compounds detected in Clinopodium vulgare* subsp. *vulgare* L.

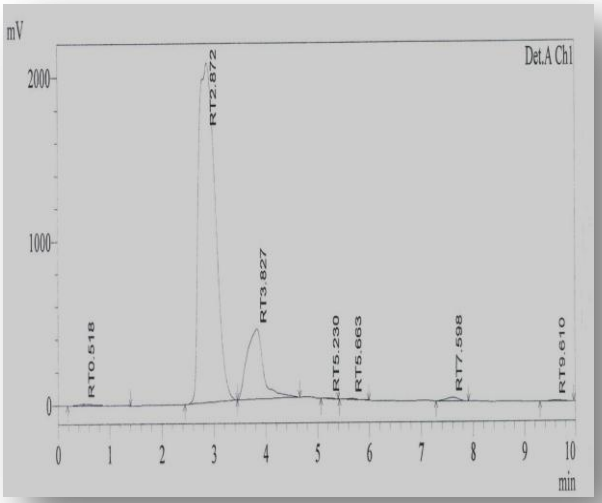

Fig. 4 :HPLC chromatograph of *the* phenolic compounds detected in *Clinopodium umbrosum* (M. B.) C. Koch.

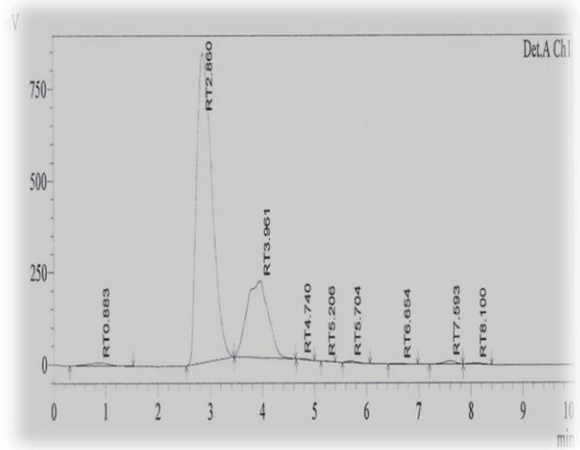

Fig. 5 :HPLC chromatograph of *the* phenolic compounds detected in *Clinopodium congstum* Boiss. & Hausskn ex. Boiss

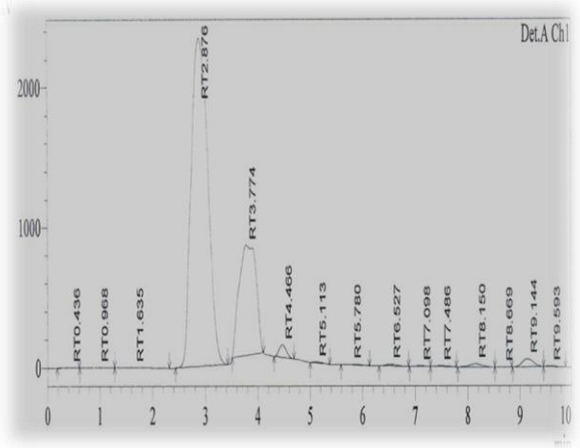

Fig. 6 :HPLC chromatograph of *the* phenolic compounds detected in *Hymenocrater longiflorus* Benth .

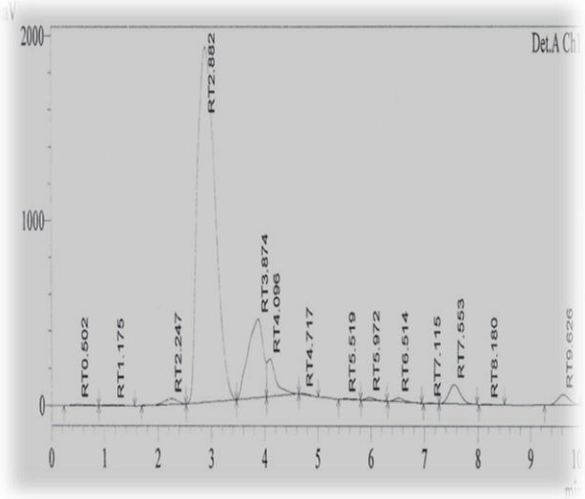

Fig. 7 :HPLC chromatograph of *the* phenolic compounds detected in *Hymenocrater bituminosus* Fish &. Mey

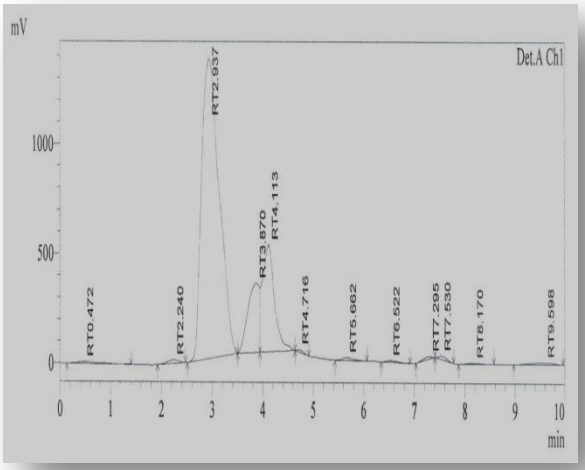

Fig. 8 : HPLC chromatograph of *the* phenolic compounds detected in *Melissa officinalis* subsp.*officinalis* L

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