Chemical profiles as chemotaxonomic tools for some species in Fabaceae in Iraq

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Abstract

Flavonoid contents were investigated in alcoholic extract of fresh leaves in some species belong to family of Fabaceae grown in Iraq which collected randomly from various fields in Hillah city. Flavonoids compounds were isolated by using Thin Layer Chromatography (TLC) in n-Butanol: Acetic acid: Water (BAW) and Ethyl acetate: Methanol: Water (EMW) solvents systems. These compounds were classified according to their Retention factor (R_f) value, color under Ultraviolet (UV) and some references.

TLC profiling of leaves extract give an idea about the presence of various phytochemicals. Different R_f value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals.

Key words: Fabaceae, TLC technique, Flavonoid, BAW, EMW, Mimosoideae, Caesalpinoideae, Papilionoideae

Introduction

Chemotaxonomy is the systematic study of chemical variation between plant taxa. Evidence of chemical variation has essentially been used for classification purposes ever since 'folk taxonomies' based on certain obvious plant characteristics were instinctively employed by mankind centuries ago [1] .

During the eighteenth and nineteenth centuries knowledge in the plant field increased and some taxonomists made use of several chemical characteristics in attempts to classify plants and to demonstrate their phylogeny. However although the chemical characters they used were recognized they were manifest at ions of processes or compounds not yet completely identified [2], one of these compounds is phenolic compounds which are secondary metabolites of plants and widely distributed throughout the plant kingdom. Secondary metabolites have a structural diversity that provide color and flavor to fruits and vegetables parts. They precipitate various pharmacological and toxicological effects on living beings. Extraction of the biological plant constituents has always been a challenging task for the researchers[3]. These were accomplished by examining the influence of different extraction solvents on the presence of secondary metabolites in the extracts by thin layer chromatography (TLC). Therefore The Fabaceae (Leguminosae) family was chosen for this investigation, because of the availability of materials belonging to this plant family.

With about 700 genera and 19,000 species, Leguminosae is the third largest flowering plant family in the world, only after Orchidaceae and Asteraceae [4], whilst [5] was estimated number of species of 18000 in 714 genera, and considered it is one of the most diverse and widely distributed flowering plant families. However, only

Poaceae rivals Fabaceae in agricultural importance, although the scope of legume uses is much greater [6]. Also, [7] emphasis that legumes have been cultivated for thousand years and played an important role in the traditional diets of many regions throughout the world

Some taxonomists regard legumes as three separate but closely related families, nominated the Mimosaceae, Caesalpiniaceae and Papilionaceae [8]. However, legumes were also viewed as a single large family, Leguminosae, largely based on their pods fruits [9]. The family Leguminosae was traditionally subdivided into three subfamilies, Mimosoideae, Caesalpinioideae and Papilionoideae [10], primarily on the basis of flower morphologies [11]. The two classification schemes are in fact similar in that they both distinguish three distinct but closely-related groups and they differ only on the taxonomic rank of these three groups [12] .

One of the aims of this research is to determine if the TLC method could differentiate between closely related species of the same family. This study was conducted in order to determine the effectiveness of this technique as a tool to identify unknown plant.

Materials and Methods

Preparation of Plant Material:

The aerial parts (fresh leaves) of different species in leguminosae family were collected randomly from various fields in Hillah city in the month of June 2014. These samples were identified according to morphological characteristic in flora of Iraq [13] and listed in table (1). The leaves samples were transported at home for cleaning by washing with tap water to remove dust and insects, then dying the sample under shade at room temperature for 2-3 days, after completing dying taking the sample to grind by kitchen grinder for 20 second, Finally the leaves sample were prepared as a powder form by using blender machine .

Ethanolic Extract:

According to [14] , (2-3) g of each plant powder was extracted for 24-48 hours with (20-30) ml of 70% (v/v) aqueous ethanol at room temperature. Filtered the extracts by using filter paper (Whatman No.1) . Then, the filtrate concentrated to an appropriate size by Air Dryer in order to get rid of the alcohol. added Petroleum Ether to the filtrate as much as its size with shake the mixture well, Put it in a separating funnel and left until it is separated into two distinct layer. Pulled the lower layer of the bottom of the funnel and left glass bottles containing them open for a few minute to concentration the extract even up to almost half its size and thus be ready to separation process

Preparation of the plates:

The adsorbent used for thin layer chromatography was silica gel G 60 (20×20)cm. The precoated TLC plates (Merk, Germany) were heated in an oven for 30 minutes at 110°C for activation. Draw a fine pencil line with left a distance of 2 cm between the sample and the other as well as the left and the same distance from the top and bottom of the plate. By capillaries tube used to put 10-15 drops of each extract on specific points of the plate with a note drying droplets by air dryer after placing every drop.

Developing solvent system

Two solvent systems were prepared in this research , first system component of the (n-Butanol: Glacial acetic acid: Water (BAW) (4: 1: 5) which put in a separating funnel after shake it well, then leave the mixture until separate into two layers, where it took the top layer and used in the work. The second solvent system component of

the Ethyl acetate: Methanol: Water (EMW) (5 : 1.1 : 1). Quietly, Put plates in a glass aquarium containing solvent and cover with a glass cover tightly then left for a period of 8-9 hours without moving. After development of plates, they were air-dried and Ultraviolet platelets examined under a wavelength of 365 nm and identified spots separated after a determined in pencil and recorded colors for each spot. R_f values were calculated. Spots were visualized by exposure to ammonium vapor reagent to find different compounds present in the extract. Some compound were diagnosed on the basis of some of the available sources such as [14], [15] and [16] after compliance with the values and R_f colors under UV and ammonia

Results

The study confirmed the presence of variations in distribution of chemical compounds between the studied species with respect to the numbers or types of compounds in both solvent systems, as shown in Figures (1, 2, 3, 4 and 5) and Tables (2 ,3).

In general, Leaf alcoholic extracts revealed (26) chemical compounds in (n-Butanol: Glacial acetic acid: Water (BAW) (4: 1: 5) solvent, while recorded (23) compounds in Ethyl acetate: Methanol: Water (EMW) (5 : 1.1 : 1) solvent represented in figures (1 & 2) which described a method of distribution of these compounds on the plates chromatography, after gave a number for each compound in both systems for the purpose of distinguishing between a compound and another in the case of knowledge of the R_f value own.

Table (1) Species under study with subfamilies in leguminosea family.

No.	Species	Subfamillies
1	*Albizia lebbeck* (L.) Benth.	Mimosoideae
2	*Albizia julibrissin* Durazz.	Mimosoideae
3	*Alhagi camelorum* Fisch.	Papilionoideae
4	*Bauhinia* spp. L.	Caesalpinioideae
5	*Caesalpinia gilliesii* L.	Caesalpinioideae
6	*Cassia didymobotrya* Fres.	Caesalpinioideae
7	*Cassia senna L.*	Caesalpinioideae
8	*Glycyrrhiza glabra* L.	Papilionoideae
9	*Medicago* spp.	Papilionoideae
10	*Melilotus officinalis* (L.) Pall.	Papilionoideae
11	*Vicia faba* L.	Papilionoideae
12	*Vigna* spp.	Papilionoideae

TLC profile indicated a difference in the number of chemical compounds in each species under study, when a solvent phase of BAW were used, a Maximum number of compounds was (10) compounds in *Albizia julibrissin* and a Minimum number was (3) compounds in *Medicago* spp. while the rest of the species recorded a clear overlap represented by (8) compounds in *Albizia lebbeck* as a nearest to another species of genus *Albizia* and (7) compounds in genus *Cassia, Caesalpinia gilliesii, Melilotus officinalis* and *Vicia faba*, (5) compounds in two species; *Bauhinia* spp. and

Glycyrrhiza glabra but the species *Alhagi camelorum* and *Vigna* spp. have (4) compounds. On other side, number of chemical compounds in the EMW solvent represented in *Vicia faba* as the higher species by containing (7) compounds while *Glycyrrhiza glabra* as the lower species which have only (2) compounds but other species overlapped with each other, for example (6) compounds in *Albizia lebbeck*, *Caesalpinia gilliesii and Melilotus officinalis*, (4) compounds in *Medicago* spp. and *Vigna* spp. while other species have (5) compounds (Figure 3).

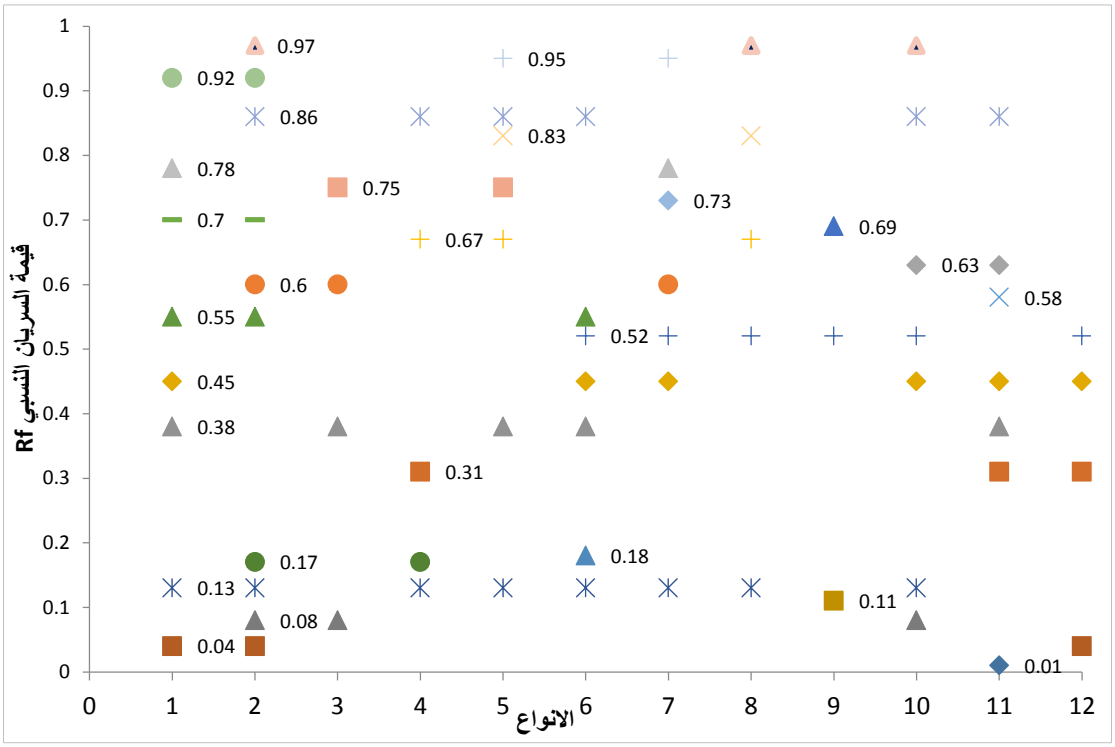

Figure (1) Distribution of Flavonoid and phenolic compound on TLC plate in leaves extract for species in BAW solvent system.

Figure (4) is shown that the phenolic compound number (5) characterized its sovereignty in leaf extract in BAW system nearby (8) species, followed by two compounds (10 & 23) in (6) species, compared with the compounds No. (1, 4, 7, 13, 17, and 19) were limited in one species as *Vicia faba*, *Medicago* spp., *Cassia didymobotrya*, *Vicia faba*, *Medicago* spp., and *Cassia senna* respectively. In contrast in EMW solvent system, Figure (5) the phenolic compounds number (1, 5, 7, 10, 12, 15 and 18) appeared in singular species like *Cassia senna*, *Alhagi camelorum*, *Vicia faba*, *Alhagi camelorum Cassia senna* and *Caesalpinia gilliesii* respectively, but a large number of species approximately (7) species were recorded in each compound (3& 22), followed by two compounds (8 & 21) in (5) species while other species overlapped between these compounds.

Unfortunately, the lack of standards at the researcher therefore not diagnose all chemical compounds in leaf extract for both solvent systems with the exception of some compounds. The most diagnostically important information in TLC analyses such as, colouration and R_f values of compound after exposed to ultra-violet and vapor ammonia then compared it with the availability of sources whilst the other

compounds maintain undiagnosed gave number and registered by the qualities of color (Table 2 and 3).

Table (2) Characteristics of phenolic and flavonoid compounds in leaves extract of the species under studied within legume family in BAW solvent system.

No.	R_f value	Coloration under ultra-violate	Diagnostic
1	0.01	Bluish green	
2	0.04	mauve	
3	0.08	mauve	Lucenin
4	0.11	Bluish green	
5	0.13	Light blue	
6	0.17	Pale brown	
7	0.18	Dark purple	
8	0.31	Blue-Bluish green	
9	0.38	Pale brown	
10	0.45	Pale brown- yellow	3-Rutinoside
11	0.52	yellow -Pale yellow	
12	0.55	light-Dark brown	3-Galactoside
13	0.58	Pale yellow	3-Glucoside
14	0.60	light brown	
15	0.63	Dark purple	
16	0.67	light brown	
17	0.69	mauve	
18	0.70	light-Dark brown	3-Arabinoside
19	0.73	Light blue	
20	0.75	light-Dark brown	
21	0.78	Pale brown- yellow	Luteolin
22	0.83	bright yellow	Kaempferol
23	0.86	brown- Orange	
24	0.92	light-Dark brown	Aglycon-Coumarin
25	0.95	Light Orange	
26	0.98	Orange	

Table (3) Characteristics of phenolic and flavonoid compounds in leaves extract of the species under studied within legume family in EMW solvent system.

No.	R_f value	Coloration under ultra-violate
1	0.02	Pale yellow
2	0.03	mauve
3	0.05	Greenish yellow
4	0.08	Mauve- milky
5	0.09	Light Orange
6	0.13	pale Milky
7	0.18	Greenish yellow
8	0.20	Mauve
9	0.25	Mauve
10	0.31	Dark brown
11	0.39	Mauve
12	0.41	Pale brown
13	0.47	Pale brown
14	0.51	Pale yellow
15	0.57	Light Orange
16	0.61	Pale yellow
17	0.62	Orange
18	0.63	Dark purple
19	0.67	Mauve- Pale yellow
20	0.77	Pale brown- Pale yellow
21	0.89	Pale-Dark brown
22	0.95	Dark brown
23	0.98	Pale-Dark brown

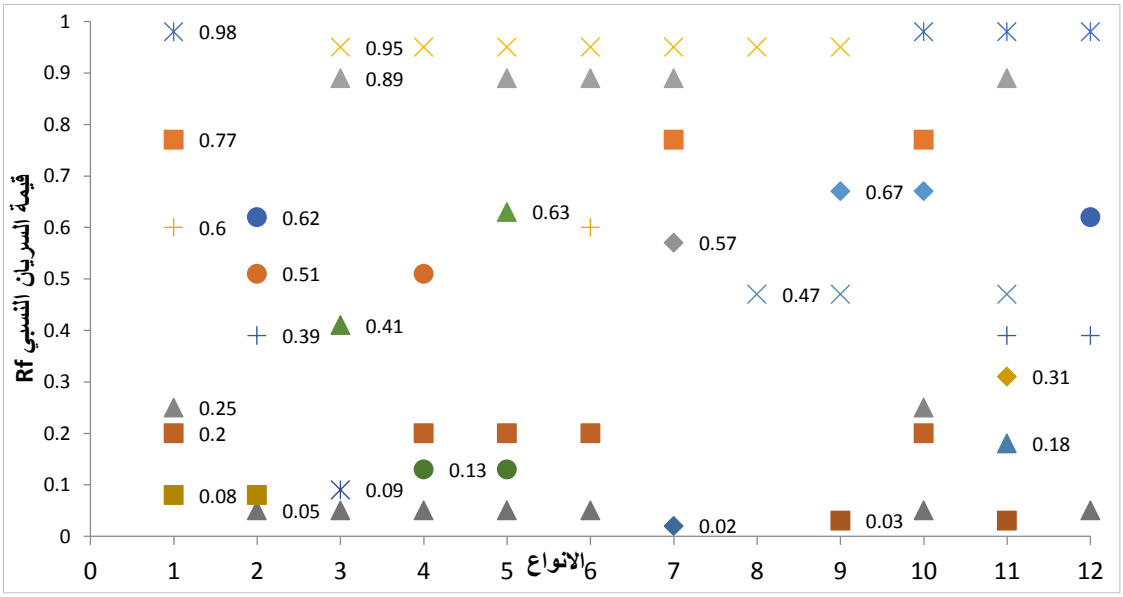

Figure (2) Distribution of Flavonoid and phenolic compound on TLC plate in leaves extract for species in EMW solvent system.

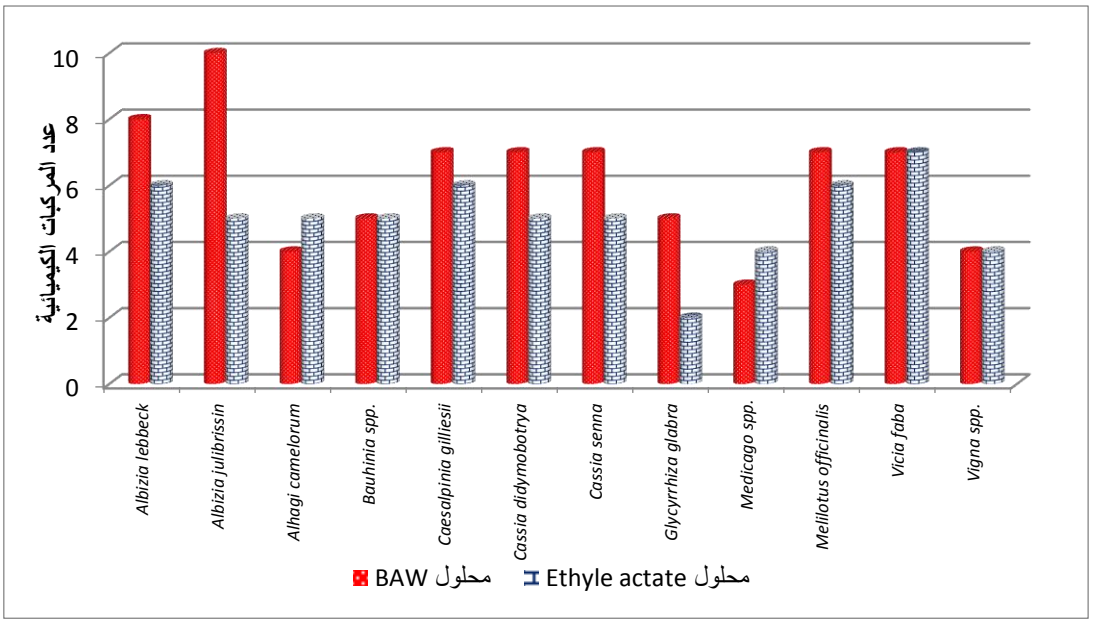

Figure (3) Number of Flavonoid and phenolic compound in leaves extract in BAW and EMW solvent systems

The results of this investigation reflect the true that R_f value of ethanol extract for different species within Fabaceae in BAW mobile phase is different from its R_f value in EMW mobile phase. The corresponding R_f value of various secondary metabolites were recorded in Table (2 & 3). The current study was able to diagnose a number of compounds depending on R_f value and color such as Lucenin was appeared as mauve in color in presence of ammonia fuming under UV light. The R_f value of this flavonol was measured as 0.08 in BAW solvent system and recorded in *Albizia julibrissin*, *Alhagi camelorum* and *Melilotus officinalis.* In addition The R_f value was measured as 0.45 and reflexed Pale brown- yellow color was diagnosed as 3-Rutinoside found in five species like *Albizia lebbeck*, , *Cassia* spp., *Melilotus officinalis*, *Vicia faba* and *Vigna* spp. Furthermore, 3-Galactoside which a various chemical constituents gave a light-dark brown color under UV light and 0.55 R_f value recorded in genus *Albizia* and *Cassia senna* but *Vicia faba* has unique compound of R_f value 0.58 and appeared as pale yellow called 3-Glucoside. As well as the possibility of isolating genus *Albizia* from the other species under study by having 3-Arabinoside and Aglycon-Coumarin which characterized as light-dark brown color with 0.70 R_f value and light-dark brown color with 0.92 R_f value respectively.

Also, The study had the diagnosis of Luteolin compound which a pale brown-yellow with 0.78 R_f value in *Albizia lebbeck* and *Cassia senna*. Likewise Kaempferol (Flavonol) was appeared as bright yellow in color in presence of vapor ammonia under UV light. The R_f value of this flavonol was measured as 0.83 in BAW solvent system recorded in *Caesalpinia gilliesii* and *Glycyrrhiza glabra*. Comparison with the R_f value of the different compounds present in the extract was found to be 0.01, 0.04, 0.11, 0.13, 0.17, 0.18, 0.31, 0.38, 0.52, 0.60, 0.63, 0.67, 0.69, 0.73, 0.75, 0.86, 0.95 and 0.98 in BAW solvent system and all compounds in in EMW solvent system, remained undiagnosed because the lack of standards and information from any study has been held in Iraq.

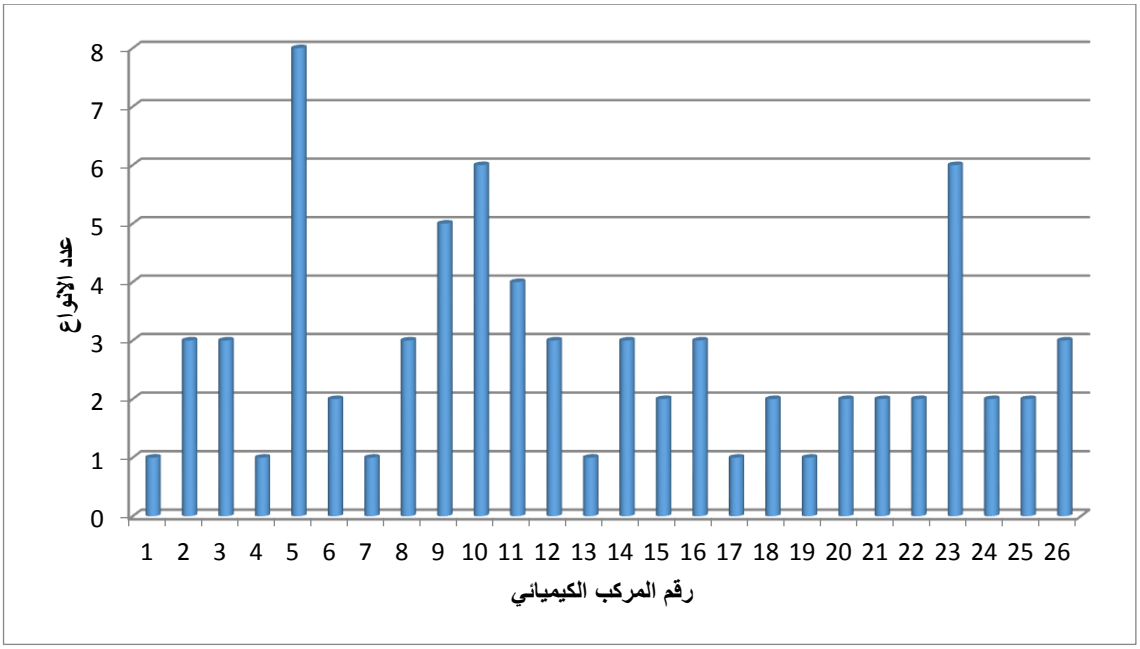

Figure (4) Number of species which have Flavonoid and phenolic compound in leaves extract in BAW solvent system

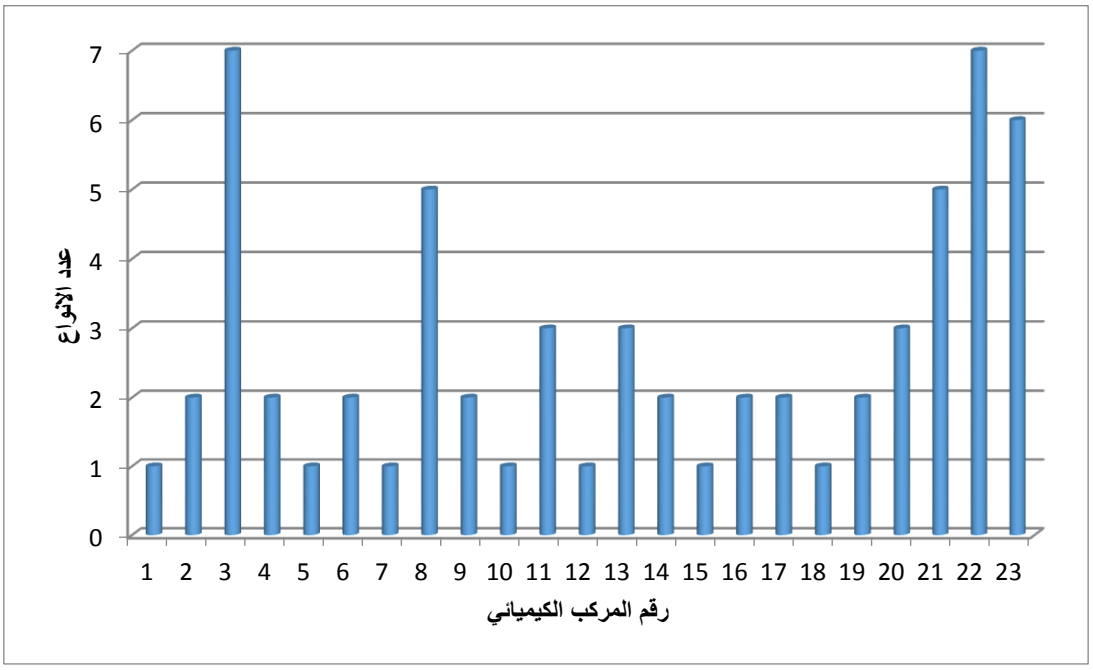

Figure (5) Number of species which have Flavonoid and phenolic compound in leaves extract in EMW solvent system

Discussion

TLC is generally regarded as a simple, rapid, and inexpensive method for the separation, tentative identification, and visual semiquantitative assessment of a wide variety of substances. In recent years, TLC has come to rival HPLC and GC in its ability to resolve complex mixtures and to provide quantitative results [17] .

Large numbers of plants can easily and rapidly be surveyed for flavonoids using paper thin- layer, or one or two dimensional chromatography and in recent years many useful results have been a achieved [18]. In addition [2] indicated that the flavonoids are one of the most useful taxonomic markers for a two reasons; Firstly they demonstrate a wide range of chemical structures which have a demonstrable genetic basis for their variation they are chemically stable, so that analysis of material can be done years after the material is collected. Secondly, they are easily isolated and identified even from small a mounts of plant material and they can be used at all taxonomic levels in most groups of plants. In the Fabaceae, is distinguished by certain characteristics such as flower color, pod size, pubescent state, vegetative organs, stem structure, and growing type, with numerous variations from other species belonging to the section [19], Results confirmed the identification solvent system is necessary to know the type and the number of compounds; This is shown in leaf alcoholic extracts when revealed (26) chemical compounds in (BAW) solvent, while recorded (23) compounds in (EMW) solvent, The reason may be attributed to Polarity. [3] pointed for extraction, the solvent is chosen as a function of the type of flavonoid required. Less polar flavonoids (*e.g.* isoflavones, flavanones, methylated flavones, and flavonols) are extracted with diethyl ether or ethyl acetate while flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol-water mixtures. [20] and [21] explained many factors, such as solvent composition, time of extraction, temperature, pH, The polarities of the polyphenols and particle size, may significantly influence in type and number of compounds in leaf extracts.

As well as, the investigation proved that R_f value of ethanol extract for different species within Fabaceae in BAW phase system is different from R_f value in EMW phase system. As the various chemical constituents gave different R_f values in different solvent systems. An idea about the polarity of various chemical constituents is also obtained while performing TLC analysis. [17] indicated the R_f value of the solute will increase with an increase in the solvent strength parameter. A solvent that has a high solvent strength parameter on one sorbent, such as silica gel, may have a different solvent strength parameter on a different sorbent.

The current study, in spite of lack of standard but they were able to clarify the relationship between species within Fabaceae in terms of the convergence or divergence from each other. Alcoholic extract of leaves observed possibility of isolating genus *Albizia* which belong to Mimosoideae subfamily from the other species under study by having 3-Arabinoside and aglycon-coumarin. In addition recorded the phenolic compound that called Rutinoside in *Cassia* species. It was indicated that similarities between closely related species, For this reason, The TLC technique is not be suitable for differentiation between closely related species. Therefore, it can only be used to identify species belonging to different families and genera .

A furthermore,[22] emphasis the existence of Kaempferol in most of the species belonging to the family of leguminous and appeared as bright yellow with 0.83 R_f value in BAW solvent system such as *Caesalpinia gilliesii* and *Glycyrrhiza glabra*. On the other side eight from thirteen species (two from Mimosoideae, four from Caesalpinioideae, two from Papilionoideae) are participated in phenolic compound

was measured R_f value as 0.13 and appeared light blue color under UV light in spite of belong different subfamilies within Fabaceae. These results agreed with [23] when he pointed to different plants sometimes contain substances which, although belonging to different types of chemical compounds, appear to be biosynthetically analogous. Such plants probably contain similar enzyme systems and the compounds which they produce may indicate a relationship between the plants**.** [24] also confirmed the presence of flavonoids, isoflavones and triterpenes in most leguminous species.

In general, it is known that chemical diversity arises from ecological diversity. However, the cause of chemical differences in some species is genetic diversity rather than ecologic diversity [25], As well as [26] revealed the same species growing in widely different habitats may drift from the original genetic makeup as a mechanism of adaptation, Therefore, same plant species that have been reproducing in different environments may have different chemical profiles. For this reason The environment has little effect on chemical composition of plants except if treated with insecticide. However, the genetic variation of a plant is more important.

Thus, The reason for the compatibility and incongruity between environmental, chemical and molecular evolution needs to be further studied. To solve this problem, there is a need to review the interactions between genes and environmental conditions, then correlation between chemical and genetic differences in the homozygous plants

In addition, Different R_f values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts

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لمحات كيميائية كأدوات للتصنيف الكيميائي لبعض انواع العائلة البقولية في العراق

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الخلاصة

تم الكشف عن المحتوى الفلافونيدي في المستخلص الكحولي للأوراق الطرية لبعض الانواع التي تعود الى العائلة البقولية النامية في العراق والتي جمعت عشوائيا من حقول مختلفة من مدينة الحلة. اذ عزلت المركبات الفلافونيدية باستخدام صفائح الكروموتوغرافيا الرقيقة Thin Layer Chromatography (TLC) باستعمال نظامين للمذيبات وهما n-Butanol: Acetic acid: Water (BAW) و Ethyl acetate: Methanol: Water (EMW) وقد صنفت المركبات اعتمادا على قيمة عامل الاعاقة Retention factor (R_f) value واللون تحت الاشعة فوق البنفسجية Ultraviolet (UV) وبعض المصادر.

ان انماط المستخلصات الكحولية على اوراق TLC اعطت فكرة عن وجود المواد الكيميائية النباتية. وان اختلاف قيم عامل الإعاقة للمواد الكيميائية المختلفة يتعلق بعامل القطبية وبالتالي اختيار المذيب المناسب لفصل المواد الكيميائية النباتية.

الكلمات المفتاحية: العائلة البقولية، تقنية صفائح الكروموتوغرافيا الرقيقة، الفلافونيدات، المذيب بيوتانول:حامض الخليك الثلجي: الماء ، المذيب اثيل اسيتيت: ميثانول: ماء، عويئلة الميموزة ، عوئيلة شوارب الملك ، عويئلة الفراشية