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Article

Comparative Chemical Study For Species of The *Eryngium* genus

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ABSTRACT

Five species of *Eryngium* L. (Apiaceae) native to Iraq-Eryngium glomeratumLam, Eryngium creticum Lam, *Eryngium thyrosoideum* Boiss, *Eryngium billardieri* Lam., and L. *Eryngium campestre*—are the focus of this comparative chemical taxonomy. Chemical analysis revealed that the five species within the genus could be distinguished by their unique chemical signatures thanks to applying the GC Mass Spectrometer (GC-MS) technique to their secondary metabolic components. Species shared specific molecules but had distinct chemical make-ups in others. The chemical properties of the species in the genus were used to calculate Euclidean genetic distance values; the highest value was found to be 5.1962 between *E.creticum* and E.billardieri, and the pair with the smallest value of Euclidean distance (3.70) was *E. campestre* and *E. billardieri*. The clustering analysis was displayed in the phylogenetic tree. The species can be classified into three broad categories along a single Euclidean axis, as indicated by chemical evidence that corroborated the result of Principal Component Analysis (4.9). (PCA). The studies also demonstrated the ability to differentiate and identify evolutionary links across species by showing the independence of species in specific isolated chemicals.

Keywords: *Eryngium*; genus; Chemical taxonomic; *Lam Eryngium*, (GC-MS) technique.

INTRODUCTION

Since the beginning of creation and until now, plants have been the primary source to meet human needs in food, housing and clothing [1]. Then, it classified them according to those needs, as plants constituted the most crucial aspect of human life since then, so man began studying them in a comprehensive study. With the progress of human perception and concepts, after the development of plants and the multiplicity of their types, and with the different environmental and climatic conditions, The interest of man began to focus on how to divide and arrange those plants in a way that makes it easy for him to benefit from and simplifies his use in his life [2]. The genus *Eryngium* is a herbaceous annual, usually perennial (perennial in Iraq) [3], rarely shrubs, leaves divided or whole, usually prickly or ciliary, flowers with dense heads surrounded by foliage, petals Deeply grooved with a tightly curved lobule. The petals are often white or colored. Fruits are ovate, oblate, and sometimes semi-spherical. More than 230 species of plants belong to the genus Eryngium, which is found in a variety of habitats around the globe (excluding regions Tropical and South Africa) [4], where the genus

Eryngium is the most prominent in the Apiaceae family [5]. Scientist [6] referred to the naming of the *Eryngium* species as medicinal plants in most European countries, where its roots and some of its vegetative parts were used to treat some kidney and bladder diseases [7]. It is widely spread in Greece, as blue dominates many of these lands. The roots of these plants are used in treating snakebite[8], and their flowers are used in some Arab countries as vegetables Table 9. Both scholars [10]indicated that the roots and leaves of plants of this genus are used to treat anemia colic and regulate the menstrual cycle and ascites. All living organisms contain a primary metabolism that provides plants with essential molecules (proteins, lipids, amino acids, nucleic acids and carbohydrates). Plants also produce compounds that do not come directly from photosynthesis but are derived from subsequent chemical reactions and are called these compounds with secondary metabolites 11. The data and chemical facts in the science of taxonomy are inseparable with the resumption of providing a vision of the relationship between plants by the internal structure; it is hoped to a large extent that these facts and chemical information will be able to reach Cytology and Genetics to compare the design of the elements of nucleic acids DNA. Thus, knowledge Inherited factors that help us in accurate diagnosis in isolating species and genera within the larger taxonomic orders; this desire arose among many researchers until the early nineteenth century. Hence, the chemical study is effectively helpful in plant taxonomy [12].

MATERIALS AND METHODS

The samples were collected in January 2022 from the herbarium (College of Education / Salah Al-Din University) in Erbil, where five samples of *Eryngium* species were obtained for study and testing.

Preparation of Plant Extract

The Sutar method was used in preparing the ethanolic solvent extract from the plant parts of the studied species as follows [13] :

Weigh 1 gm of the ground plant sample pre-dried by sunlight with a sensitive scale, wrap it in filter paper after recording the sample name symbol on it, and place it in the middle of the Soxolith apparatus.

Analysis using a Gas ChromatographicMass Spectrometer (GC-MS)

The alcoholic extract of the plant was analyzed by a GC-MS of the type Agelint (7820A) USA GC Mass Spectrometer, which is connected to the MS Spectrometer according to the following conditions for analysis :

1. Ultra lneit HP-5ms Column Capillary Separator 30m x 250um diameter x 0.25 um inside diameter (30m length x 250um diameter x 0.25um inside diameter) of 100% dimethyl polysiloxane that acts as an electron sniper detector.
2. The flow rate was 1 ml/min, and the carrier gas was folium gas, with a purity of 99.999%.
3. The injector temperature was 250 C.
4. GC inlet line temperature 250°C
5. The injected fluid was a volume of (1) µL
6. The temperature of the oven is programmed at 60 ° C, with an increase of 7 ° C / min, and the increase continues until it reaches 180 ° C, then increases to 280 ° C and remains stable for 7 minutes.
7. The total time is 34 minutes from starting the device until it turns off
8. The pressure inside the device was 11.933 psi
9. Scanning range 50 m/z-500
10. The injection type was Splitless

The injection type was Splitless.

The chemical compounds were diagnosed according to GC-MS mass spectrometry, and the Industrial Research and Development Organization database was used to ascertain the identity of the compound, the molecular weight, and the structure of the test components. This test was conducted in the GC-MS unit at Ibn Al-Baytar Research Center / Baghdad.

RESULTS

Color chromatography-MS analysis (GC-MS) identified 100 peaks at the top of about 3 chemical compounds in the alcoholic extract of the species under study, where each species contained 20 peaks, as shown in the figures listed below.

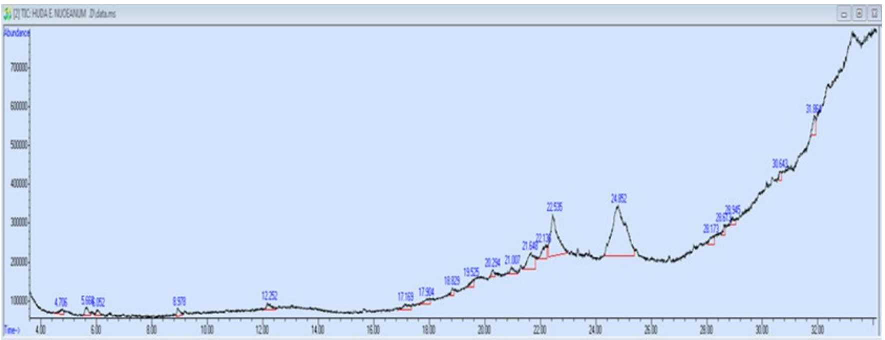

Figure 1. Stander curve for chemical compounds of the type E-glomeratum.

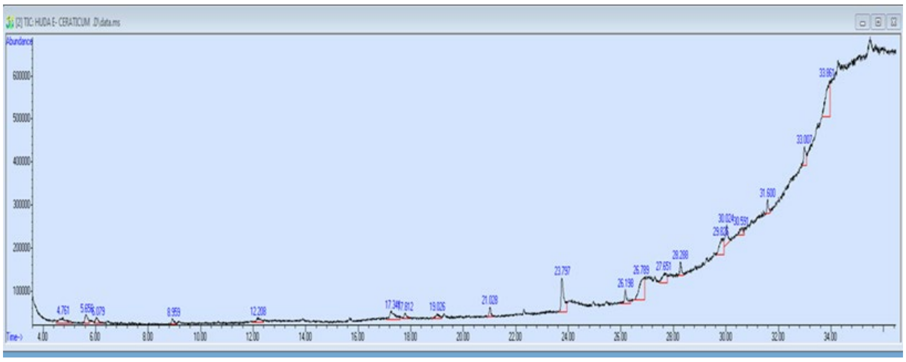

Figure 2. Stander curve for chemical compounds of type E- ceraticum.

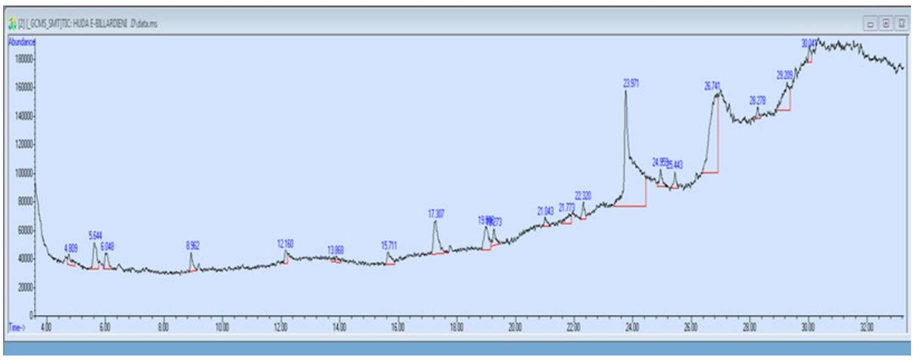

Figure 3. Stander curve for chemical compounds of the type E- billardieni

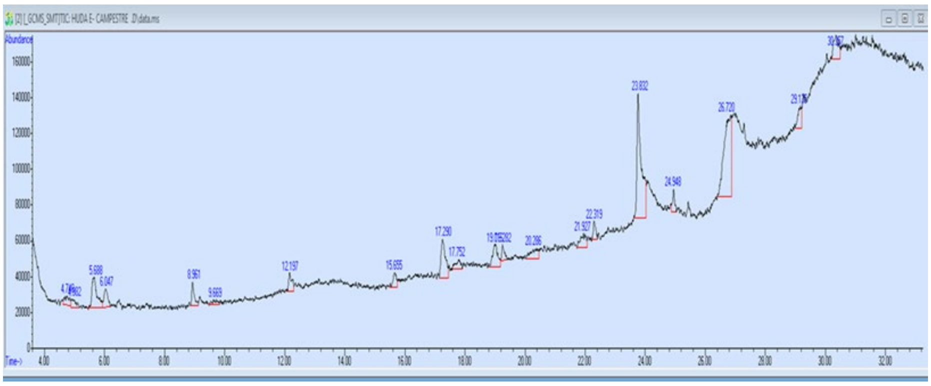

Figure 4. Stander curve for chemical compounds of the E-Campestre

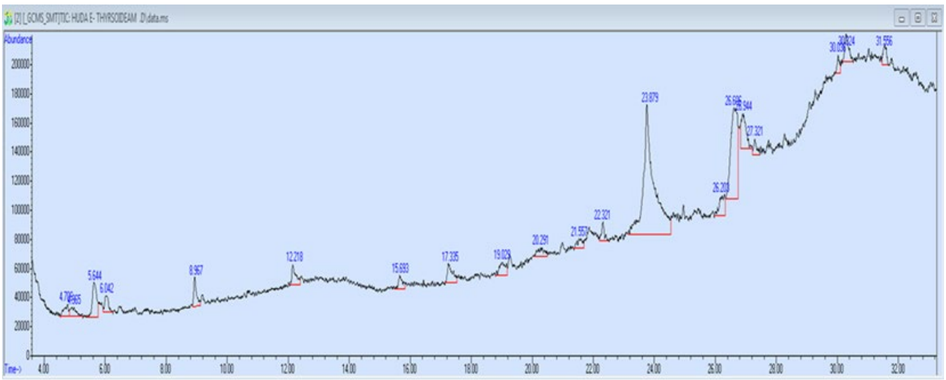

Figure 5. Stander curve for chemical compounds of the E- thyrosoideum

Table 1. indicates the common compounds between the species of the genus *Eryngium* under study based on the chemical results obtained for the species using the GC-MS technique. As it was found that *Eryngium-glomeratum* shared several chemical compounds with other species, the number reached (13) compounds, while it was unique to the other compounds, which amounted to (47) compounds. As for the species *Eryngium - creticum*, it was associated with the species under study with (16) chemical compounds, while it was unique with (44) other compounds Also, the species *Eryngium - billardieri*, when separated, showed that it shared (19) compounds with the studied species of the genus *Eryngium*, while it was isolated with several compounds that reached (41) compounds , while the type *Eryngium - campestre* showed its overlap with other types with (16) chemical compounds and its isolation with (44) other compounds Also, the type *Eryngium - thyrosoideum* contained (18) common compounds, while it contained (42) other single chemical compounds.

NO.	**Compound name**	***E.thyrosoideum***	***E.campestre***	***E.billardieri***	***E.creticum***	***E.glomeratum***
1	cyclopentadiene [c] furo [3,2:4,] furo [3-h] 2,3,6a,9-tetrahydro-1,3-dihydroxy-4-methoxy benzo[a]pyran-11(1H)-one			√		√
2	The Pterin-6-Carboxylic Acid	√	√	√	√	√
3	Chemical formula: 2,4,6-trimethyl-5,6-dihydro-1,3-dithiazine		√	√	√	√

4	N,N-Dimethylethanesulfonamide			√		√
5	1-Decanamine			√		√
6	1-Nitro-beta-d-arabinofuranosyl tetraacetate	√				√
7	Toluene, 4,7,7-trimethyl-, semicarbazone, bicyclo[2.2.1]heptan-2-one	√		√		√
8	Acetate of (S)-Z-13-methyl-11-pentadecen-1-ol			√		√
9	The oleic acid	√	√	√		√
10	Octadecanol, 2-methyl-, zz-313		√	√	√	√
11	1,6-Dimethyl-9- (1-methylethylidene) -5,12-dioxatricyclo[9.1.0.0(4,6)] dodecane-8-one	√				√
12	The cyclododecanol, 1-ethenyl-		√			√
13	Esters of 2,2-dimethylpropanoic acid and 2,6-dimethylnon-1-en-3-yl				√	√
14	A 3-(cyanoethyl) tetrahydrofuran -4-thiopyranone			√	√	
15	3-(4-nitrophenyl amino)indole	√	√	√	√	
16	1,3,5,7-tetramethyl-1-methoxycyclotetrasiloxane	√			√	
17	N6,N6Dimethyladenosine		√		√	
18	The 2-Cyclohexanone (dimethylhydrazine) -3-[4-hexenyl] -1-aci-nitro- (E,E)	√			√	
19	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	√			√	
20	1,5,6,7-Tetramethylbicyclo[3.2.0] hept-6-en-3-ylideneemicarbazide		√		√	
21	Methyl ester of 11-octadecenoic acid	√			√	
22	Acyl methyl ester of 9-octadecenoic acid (Z)	√			√	

23	Methylene trans-13-octadecenoic acid	√			√	
24	2-dodecen-1-yl(-)succinate	√	√		√	
25	Chemical formula: 1-(1,5-dimethylhexyl)-4-(4-methyl pentyl)-cyclohexane			√	√	
26	1-Dodecanamine	√		√		
27	1-Heptadecanamine		√	√		
28	Bromo-2-ethyl	√	√	√		
29	cis-Vaccenic acid	√		√		
30	[2](1,4)Anthraceno[2](2,6)pyrid ine phane-1,13-diene		√	√		
31	2-Hydroxy-1-(hydroxymethyl)ethyl ester of 9-octadecenoic acid (Z)		√	√		
32	1H-Indene,5-butyl-6-hexyloctahydro-		√	√		
33	2-Methyl-3,5-dinitrobenzyl alcohol,tert-butyldimethylsilylether	√	√			
34	3,6-Bis-dimethylaminomethyl-2,7-dihydroxy-fluorene-9-one	√	√			

Table 1. Compounds common to the species of the genus *Eryngium*.

The results shown in Table 2. refer to the values of genetic dimensions amongst the class of the genus Eryngium in Iraq, based on the chemical study of the species under study using GC-MS technology. It was found that the highest Euclidean distance was (5.196) between the two species E- billardieri and E- creticum and that the lowest Euclidean distance was (3.7417) between the two species under study, E- billardieri and E - campestre.

Types	*E.glomeratum*	*E.creticum*	*E. billaedieri*	*E. campestre*	*E. thyrosoideum*
E.glomeratum	0				
E.creticum	5	0			
E. billaedieri	3.8	5.1962	0		
E. campestre	4	4.5826	3.7417	0	
E. thyrosoideum	4.2426	4.7958	4.4721	4.2426	0

Table 2. The Euclidean genetic distance for species of Eryngium is based on chemical indicators.

Figure 6 shows the cluster distribution scheme based on the results of separating chemical compounds using the GC-MS technique for the species under study in Iraq. The first significant clade included *E.creticum*, the secondary clad included *E. campestre* and *E. thyrosoideum*, and finally, the combined secondary clad of the double species. *E. billaedieri* and *E. glomeratum*.

The type clade or the secondary clade of E. thyrosoideum was considered the sister clade of the main clade of *E.creticum* with a complete cluster strength that reached 100% and an Euclidean distance of (4.9) distributed over the species. The secondary connective clades of the three species *E. campestre*,*E. glomeratum*, and *E. billaedieri*, in particular, were related and considered the sister clade of *E. campestre*, with a cluster strength of 90% and a Euclidean distance (4.3) between them.

As for the last clade, the most expensive sub-clade, the first was characterized by the two species *E. billaedieri* and *E. glomeratum*, with good cluster strength between them (70%) and with an Euclidean distance (3.7) between them, which was considered the brother clade associated with the clades of type *E. campestre* and with an outstanding reliability (80%). With an Euclidean distance of 3.8) between them.

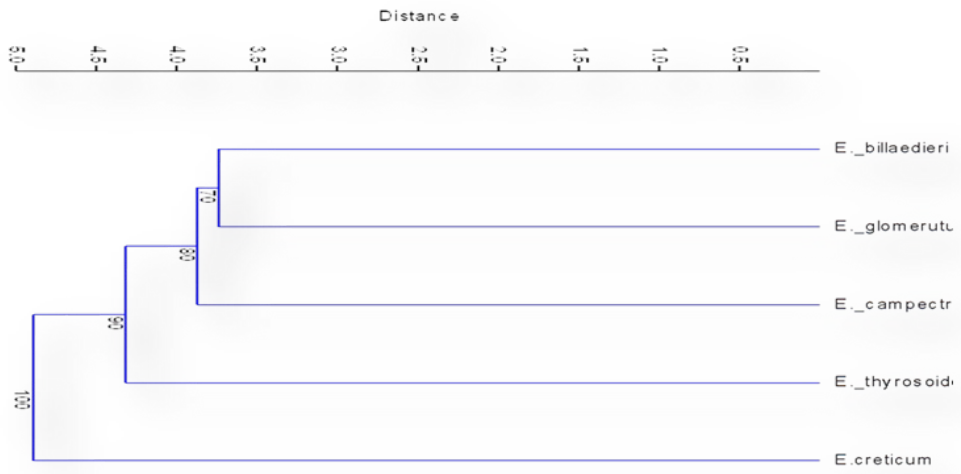

Figure 6. Genetic kinship tree of the genus *Eryngium* in Iraq, according to the UPGMA

Method (14), depending on the results of the chemical study, Figure 7, a precise match with the results of the genetic kinship tree in Figure (6), as the distribution results showed the matching of the groups that make up the genetic kinship tree based on chemical indicators, which show their distribution in two groups or significant clades, the first group included the clades that contain the two species *E. billaedieri* And *E. glomeratum*. In contrast, the second group, or the second major clad, included *E. thyrosoideum*, *E. campestre* and *E. creticum* under study.

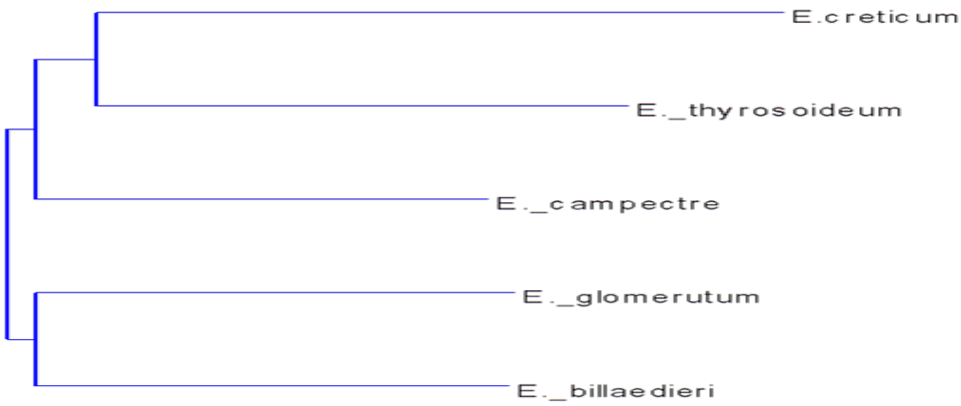

Figure 7. Genetic tree of Eryngium species based on chemical indicators by Neibor joining.

Table 3. indicates species occurrence in two main components, depending on the chemical indicators amongst them done Principal Component Analysis (PCA) using the PAST program. Component 1 recorded a variance rate of 38.59%, while Component 2 gave a genetic variance rate of %25.699, totaling 64.289% based on chemical indicators.

PC	Eigenvalue	% variance
1	3.78184	38.59
2	2.5185	25.699
3	1.93831	19.779
4	1.56136	15.932

Table 3. PCA component analysis based on chemical indicators.

DISCUSSION

Common chemical compounds between species

When chromatographic analysis was performed with a GC-MS device, it was possible to separate species from the genus Eryngium based on the number of separated chemical compounds, although there was overlap between species. Each species under study, separated by the GC-MS device, contained 60 chemical compounds that shared several compounds while they were unique to others. Furthermore, this case constitutes an evolutionary phenomenon of importance, as the participation of species of the genus in certain compounds confirms the existence of a common evolutionary link between the species in terms of chemical characteristics, and this reflects its return to one common ancestral origin, and this was confirmed by the results of previous studies on certain plant families on the taxonomic importance For chemical compounds in distinguishing plant groups, including a study [15]. The species of the genus *Eryngium* growing in Iraq showed significant and many variations in terms of their content of secondary metabolites, as many compounds were extracted, purified and separated using GC-MS technology, which reached a total of (300) compounds in all studied species, and the differences were returned. This chemical is one of the auxiliary taxonomic indications, which is on the side of the importance and one of the essential auxiliary factors in resolving much confusion and overlap between closely related species, which is difficult to distinguish between them except by using the results of different supporting studies in addition to the current molecular studies in agreement with what was reached Kremer [18] who pointed out the importance of chemical separation of phenolic compounds and essential oils in isolating and characterizing *Eryngium* species from each other.

Genetic dimension values based on chemical indicators

The results shown in Table (2) refer to the values of genetic dimensions amongst the species of the genus *Eryngium* in Iraq, built on the results of the chemical study of the species under study using GC-MS technology. It was found that the highest Euclidean distance was (5.196) between the two species *E- billardieri*and *E- creticum*, and that the lowest Euclidean distance was (3.7417) between the two species under study, *E- billardieri*and *E - campestre*. The results of the current study showed that the Euclidean distance between the sexes was ok.

Genetic kinship tree among species of the genus Eryngium based on chemical evidence.

The effects of the current study bared that the species belonging to the genus *Eryngium* fall into one leading group despite the species being separated from each other; as for the affinity with which some species came from their isolation and distribution within secondary clades, it confirms their introversion into one genus belonging to the family Apiaceae 16. The similarity in the chemical characteristics under study is reinforced by the ordinary ancestral regression of the genus types, in addition to the fact that these species occupy one geographical area with very close environmental conditions despite their differences in many other phenotypic characteristics. However, they are genetically close to each other, which was confirmed by the chemical study [17].

Analysis of the main component of Eryngium species based on chemical indicators

The results of the PCA (Principal Component Analysis shown in Figure (7) showed a precise match with the results of the genetic kinship tree in Figure (1), as the distribution results showed the matching of the groups that made up the genetic kinship tree based on the chemical indicators, which shows their distribution in two groups or clades The first group included the clades containing the two species *E. billaedieri* and *E. glomeratum*. In contrast, the second group, or the second major clade, included *E. thyrosoideum*, *E. campestre* and *E. creticum* under study.

The Principal Component Analysis (PCA)

The PAST program. Component 1 recorded a variance rate of 38.59%, while Component 2 gave a genetic variance rate of %25.699, totaling 64.289% based on chemical indicators. This is a positive indication of the efficiency of the chemical evidence used in the study in diagnosing plants and the differences between the species under study and their ability to estimate the magnitude of variations at the molecular level and at the level orthogonal to the two axes and their distribution through all the studied chemical indicators, which reflects the reality of these indicators and their comprehensiveness in plant cells [18]. From all of the above, it became clear that it is possible to divide species, clarify the genetic Euclidean dimensions and clarify the kinship and divergence between species depending on the indicators of the chemical study, which may not strongly support other aspects such as anatomical and molecular studies. However, fortunately, it has proven its limits in the separation and distinction and finding evolutionary links between the species of the genus *Eryngium* in Iraq.

CONCLUSIONS

From the current study, it became clear that it is possible to divide species, clarify the genetic Euclidean dimensions and clarify the kinship and divergence between species depending on the indicators of the chemical study, which may not strongly support other aspects such as anatomical and molecular studies. However, fortunately, it has proven its limits in the separation and distinction and finding evolutionary links between the species of the genus *Eryngium* in Iraq.

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