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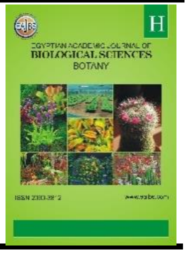

Chemical Classification of some Species *Euphorbia* in Central and Northern Iraq

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ABSTRACT

Plant samples of the studied species of the genus Euphorbia were collected from several provinces, including central and northern Iraq, during the 2021-2022 season, as these species included: *E. macroclada*, *E. hirta*, *E. falcata*, *E. Craspedia*, *E. helioscopia*, *E. peplus*, *E. spp*, *E. spp* (2) and *E. denticulata.* The results showed that the phenolic compounds that were detected were nine: Gallic acid, Quercetin, chlorogenic acid, Caffeic acid, Syringic acid, Ellagic acid, Ferulic acid, Kaempferol, and Myricetin. The studied plant species were divided into several groups according to the total content of phenolic compounds, namely: a group with a high content of phenolic compounds in which the total concentration of phenols was more than 1000 mg/L, including type E.spp, and a group with a medium content of phenolic compounds, the total concentration of the compounds ranged Phenolic content ranged between 700-900 mg/L including the species *E. Craspedia, E. peplus* and *E. spp*(2), and a group with a low content of phenolic compounds in which the total concentration of phenolic compounds was less than 600 mg/L included the species *E. macrolaeda* and *E. Craspedia*, *E. hirta*, *E. falcata*, and *E. Helioscid.*

INTRODUCTION

Phytochemicals are secondary metabolites in one or more parts of medicinal plants that have the ability to perform a specific physiological action on the human body (Acharya and Vaidya, 2017). Phytochemicals found in plants, such as steroids, glycosides, flavonoids, alkaloids, amino acids, saponins, tannins, etc., provide a great source of new and promising drugs (Saswade, 2019). Essentially, phytocomponents from all parts of the plant body including the root, stem, leaves, bark and seeds (Nandagoapalan *et al.* (2016) have evolved to produce bioactive compounds that serve to protect against various diseases and stress conditions (Thilagavathi *et al.*, 2015). The interaction between phytochemicals and the plant's bioactive machinery is important for understanding the production of molecules with specific activities for the treatment of many communicable and chronic diseases (Pandey *et al.*, 2013). Secondary metabolites, which are biosynthesized from primary metabolites and are an important source of many nutrients, are abundant in plants belonging to the Euphorbiaceae family (Al-Snafi, 2017).

Phytochemicals and nucleic acids provide valuable tools for the identification of various taxa and their placement in the natural classification system. Chemical compounds are broadly categorized as visible adjectives, fine and macromolecules. Small and large chemical molecules provide important classification tools. Molecular properties are small in size and include phenolic constituents, alkaloids, terpenoids, free amino acids, fats, oils, and glycosides. The macromolecules are composed of proteins and nucleic acids. Although chemical properties provide important taxonomic tools, some of them such as alkaloids and proteins are subject to change with respect to changing environmental conditions or developmental stages. Therefore, plant parts of the same physiological age should be used for comparative analysis (Sharma, 2013).

The phytochemical properties of plants belonging to the Euphorbiaceae family include hydrocarbons, phenolic compounds (Flavonoids, lignin, coumarins and tannins), alkaloids, cyanogenic glycosides and glucosinolates; It is thus one of the most important families in terms of chemical diversity and thus has great economic potential (Seebaluck-Sandoram *et al.*, 2018). Species of the genus Euphorbia are of great interest, given the wide diversity of secondary metabolites produced by this genus, many of which offer a wide range of actions against pathogens and diverse opportunities for use in scientific research with the aim of developing new pharmaceutical products (Ferreira *et al.*, 2020).

Yang *et al.* (2011) indicated that some secondary metabolites present in some species of the genus Euphorbia, such as flavonol galactopyranosides, including quercetin, Kaempferol, and Myricetin, as in *E. hirta,* exhibit many therapeutic properties. The most isolated chemicals from the genus Euphorbia are flavonoids, triterpenes, and phenolic compounds, including quercetin, which are highly effective as an anti-inflammatory, antioxidant, antitoxin, and antimicrobial (Ibrahim *et al.*, 2013). A study by Shin yang-Soo (2013) indicated that some chemical compounds such as quercetin and coumarin isolated from some species of the genus Euphorbia have important biological activity because they are used as emetics and laxatives.

The current study aimed to distinguish between nine plant species belonging to the genus Euphorbia by analyzing their content of polyphenols and the concentrations of these compounds.

MATERIALS AND METHODS

Study area and sample collection: Samples were collected from several provinces, including central and northern Iraq, starting from 5/23/2021 until 4/11/2022.

Determination of phenolic compounds: The study was conducted according to the method of the scientist (Jone, 1995) using a high-performance liquid chromatography HPLC model Shimadzu HPLC-10 (Double pump model HPLC-10A Shimadzu), of Japanese origin, and according to the following steps:

• Extraction of phenolic compounds: The extraction process was carried out by taking 15 gm of the ground and homogeneous form and adding 15 ml of chloroform and 10 ml of hexane to remove chlorophyll, turbines and fats present in the plant. It was left for 10 hours with continuous stirring, then the extract was placed in a sonar cracker for a period of time. 20 minutes at a temperature of 45 ˚C, then 25 ml of methanol was added to it, then transferred to a separation funnel, then the polar organic layer (methanol) was collected, and transferred to a rotary evaporator to obtain a dry extract, then the process was repeated three times to obtain a sufficient amount before analysis.

• Analysis conditions: The carrier phase (methanol: distilled water: formic acid) was used in a ratio of (70: 25: 5), and the separation column was (C18 - ODS) with dimensions (25 cm × 4.6 mm) to separate the phenols and an ultraviolet radiation detector (UV - 280 nm), while the flow rate of the carrier phase was (1.0 ml/min). Table (1) shows the conditions for the

HPLC device for the detection of phenols and flavonoids. The results of reading the standard solution revealed the detection of nine phenolic compounds (Gallic acid, Quercetin, Chlorogenic acid, Caffeic acid, Syringic acid, Ellagic acid, Ferulic acid, Kaempferol, and Myricetin).

Table 1: HPLC conditions for the detection of phenols and flavonoids.

Vocabulary	Separation condition
Column	separation of phenols mixture on reversed phase C-18 (150x 4.6 mm) column, 5µm particle size ID.
Mobile phase	0.7% acetic acid in acetonitrile: water (1:1) v/v
Flow rate	2 ml/min
Detector type	UV detector 280 nm
Temperature experiment	30 ˚C.
Standard Concentration	standard 50 µm /ml

The calculations were made according to the following equations:

$$\textbf{The compound conc.} = \frac{\textbf{The compound area in the sample}}{\textbf{standard space}} \times \textbf{standard conc.} \times \textbf{dilution}$$

The dilution for all samples = 2

$$\text{Percentage} = \frac{\text{Partial conc.}}{\text{Total conc.}}$$

RESULTS AND DISCUSSION

Table (2) and Figure (1) show the results of the chemical analysis for the detection of phenols using the high-performance liquid chromatography (HPLC) device in the plant samples of the studied species that are collected from the study areas. chlorogenic acid, Caffeic acid, Syringic acid, Ellagic acid, Ferulic acid, Kaempferol, and Myricetin.

Table (2) showed that Ellagic acid was not present in the species *E. hirta*, *E.falcata* and *E.peplus*, while it was present in the rest of the species, and the type E.spp recorded the highest concentration of (123.75) mg/L, while the lowest concentration was 17.61).) mg/L in E.helioscopia.

The compounds Kaempferol and Myricetin were somewhat similar in terms of presence and absence, as each of them was recorded in species *E.macrocloda, E.hirta, E.falcata, E.helioscpia* and *E.spp,* and it was not recorded in the rest of the species except for *E.helioscpia,* in which the concentration of Kaempferol reached 195.85 mg. /Liter. The results showed that Ferulic acid was recorded in *E. macroclada* and *E. spp*, while it was not recorded in the rest of the species.

Table 2: The concentration of phenolic compounds in the studied samples.

#	The species	Gallic acid	Quercetin	Chlorogenic acid	Caffcic acid	Ellagic acid	Ferulic acid	Kaempferol	Myricetin	Total
1	***E.macroclada***	120.61	-	15.09	120.77	36.50	34.71	158.14	190.88	676.702
2	***E.hirta***	225.23	189.79	24.39	31.04	-	-	194.43	122.97	787.85
3	***E.falcata***	290.47	176.46	40.86	37.85	-	-	147.35	76.34	807.02
4	***E.craspedia***	174.88	139.81	32.81	108.29	73.45	-	-	-	584.51
5	***E. helioscopia***	422.92	-	27.38	-	17.61	-	195.85	-	737.86
6	***E.peplus***	57.90	78.09	-	-	-	-	48.21	146.56	330.76
7	***E.spp***	406.23	-	-	-	112.88	98.27	197.89	230.33	1024.55
8	***E. spp(2)***	-	186.51	81.18	54-42	123.75	-	-	-	597.29
9	***E.denticulata***	324.59	-	-	-	79.41		168.68	153.34	749.86

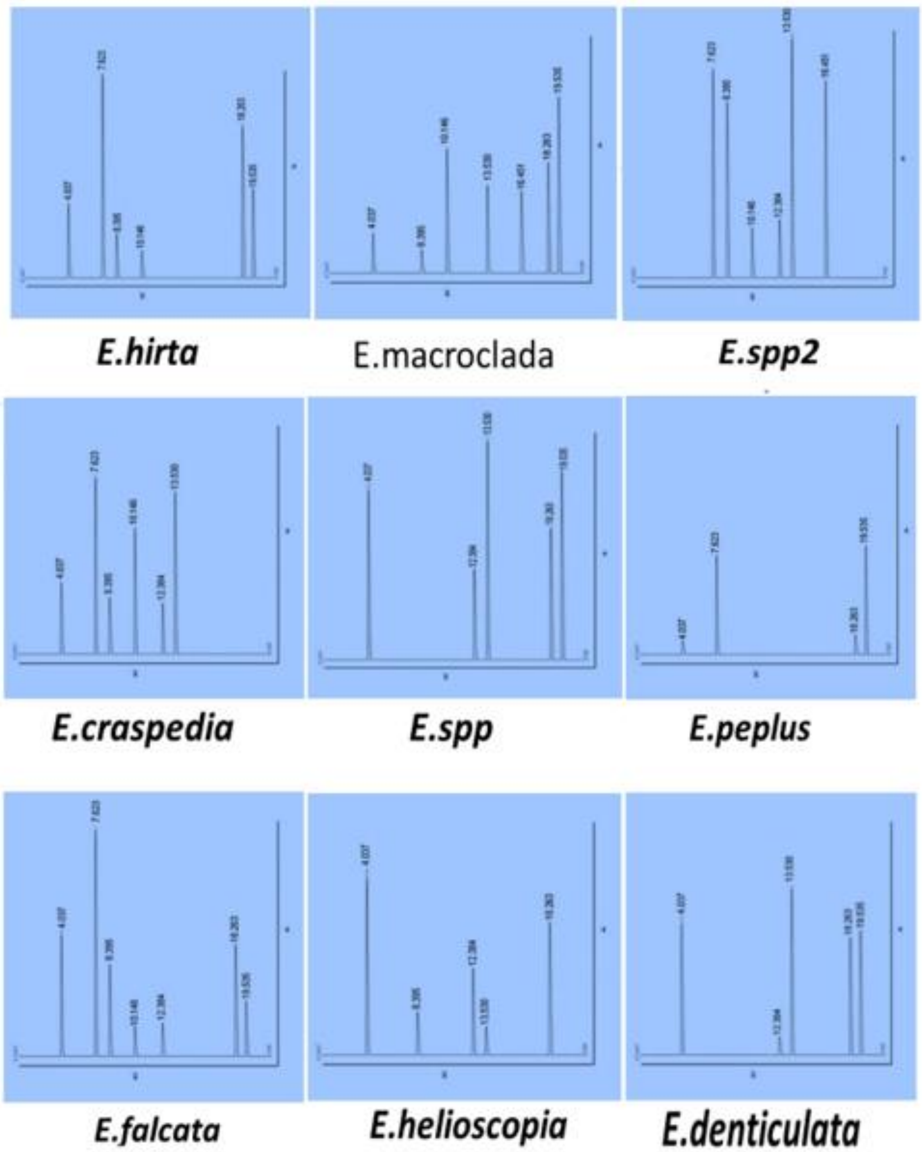

Fig. 1: The detected phenolic compounds for each plant species according to the retention time using the HPLC device.

It is clear from the results in Table (2) that there is a difference in the chemical content between the studied species, as gallic acid was recorded in all species except *E.spp* (2), and *E.helioscopia* gave the highest concentration of (422.92) mg/ L, while the lowest concentration was recorded by *E.Peplus* and reached (57.90) mg/L. The results showed the presence of Quercetin in *E.hirta, E.falcata, E.Craspediag, E.Peplus* and *E.spp* (2), and it was not recorded in the remaining species and reached The highest concentration was in E.hirta (184.74) mg/L, while the lowest concentration was (78.09) mg/L and was in *E.Peplus*. Chlorogenic acid did not differ much from Caffeic acid, as its presence was detected in *E.macrolaeda*, *E.hirta*, *E.falcata, E.craspedia,* and *E.spp* (2), and the presence of Chlorogenic acid in *E.helioscid* as well.

It is worth noting that E. macrolaeda recorded the highest concentration of Caffeic acid, which reached (120.77) mg/L, and the lowest concentration of Chlorogenic acid was (15.09) mg/L. As for Syringic acid, it was found in *E. falcata* and *E. craspedia*, and *E. helioscid* and *E. spp,* and *E. spp* (2) and *E. denticulata*. The highest concentration was recorded in E. spp (77.22) mg/L, while the lowest was (23.84) mg/L in E. spp (2).

From the foregoing, it is clear that the plant species can be divided into three groups according to the presence and absence of phenolic compounds.

1- The first group: - Species similar in that they contain four identified phenolic compounds (Quercetin, Chlorogenic acid, Caffeic acid, and Syringic acid), which are the species *E. falcata* and *E. Craspedia* and *E. spp* (2).

2- The second group: - Species that are similar in containing Kaempferol and Myricetin compounds, which are the species *E. macrolaeda* and *E. hirta* and *E. falcata,* and *E. Peplus*, and *E. spp,* and *E. denticulata.*

3- The third group: - Species that are similar in containing Ferulic acid, which is *E. macrolaeda* and E. spp.

From the current results, we note that the species E. peplus recorded the least number of phenolic compounds, amounting to only 3 (Quercetin, Kaempferol, and Myricetin), while the species *E. helioscid* and E. spp, and *E. denticulata* having 4 compounds, the species *E. hirta* and *E. Craspedia* had 5 compounds, while the number of diagnosed compounds increased to 6 in the two species *E. macrolaeda* and *E. falcata.*

Regarding the total content of phenolic compounds, it was possible to divide the species into several groups:

1- A group with a high content of phenolic compounds: it includes the type E.spp, in which the total concentration of phenols reached more than 1000 mg/liter.

2- A group with medium content of phenolic compounds: it includes the species E. Craspedia, E. peplus and E. spp (2). The total concentration of phenolic compounds ranged between 700-900 mg/l.

3- A group with a low content of phenolic compounds: *E. macrolaeda* and *E. Craspedia, E. hirta, E. falcata*, and *E. Helioscid,* in which the total concentration of phenolic compounds was less than 600 mg/l.

The present findings are consistent with Pesano *et al.* (2016) who indicated that extracts of Euphorbia *E. characias* represent a good source of natural bioactive compounds that can be useful for pharmaceutical applications as well as in the food system to inhibit the growth of foodborne bacteria and extend the shelf life of processed foods, and that the ethanolic extract of the leaves contains high levels of Quercetin. It also agrees with Ibrahim *et al.* (2013) who confirmed that flavonoids are the most isolated plant compounds from Euphorbia species, including quercetin and triple terpenes, which are antioxidant compounds that are effective against infections, toxins, microbes, and intestinal diseases. Bezerra *et al.* (2020) stated that the index of phenolic contents in Croton matourensis extracts depends on the polarity of the solvent used, in addition to the fact that phenols have a broad polarity that allows them to be obtained with different solvents, and they provided evidence on the role of these compounds in the antioxidant and antimicrobial activities shown by ethanol extracts. In general, the results of this study agree with the recommendations of Al-Atabi and Khalaf (2002), who considered chemical compounds of great importance taxonomically, and scientists found that they evolved and specialized with the evolution and specialization of plants and their parts, such as alkaloids, quinones, essential oils, saponins, phenols, anthocyanins, and crystals are among the distinguishing characteristics of a number of One of the families or groups of seed plant families, including the Euphorbiaceae family.

Conclusion:

We conclude from the results of the current study that there is a discrepancy in the quantitative and qualitative content of phenolic compounds in the nine studied plant species of the genus Euphorbia, and this indicates the possibility of adopting the chemical content as a taxonomic guide among the different plant species, especially of the genus Euphorbia.

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