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Determination of Phenolic Compounds in Some Plant Species Belonging to the Genus Lepidium of the Cabbage Family Brassicaceae in Central and Northern Iraq Using the HPLC Technique

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ABSTRACT

The chemical study included the diagnosis and estimation of phenolic content in the leaves of the six studied species, namely L.sativum Biosser and L.ruderale Biosser and L.latifolium Biosser and L.persicum Biosser and L.perfoliatum Biosser and L. aucheri: Biosser using the HPLC high-performance liquid phase chromatography technique , eleven phenolic compounds were diagnosed in the studied species, namely Curcetin, Ferulic acid, Sinapic acid, P-Comaric acid, Caffeic acid, CHlorogenic acid, cinnamon acid, Epicatechoic acid, Catechoic acid, Kaempferol and protochatechoic acid species varied among themselves in the presence or absence of compounds in the studied species, and the current study showed a clear variation in the total content of phenols in the leaves of the studied species, distinguishing type L.latifolium has the highest phenolic content at 170.841 micrograms / ml , while the lowest phenolic content was in type l leaves L.perfoliatum reached 15.666 micrograms / milliliter.

Therefore, the genetic and chemical study distinguished the species among themselves and determined their distance and proximity to each other and isolated species from other species in determining the relationship between them .

Introduction

Man has been interested in plants since God Almighty created him and his life was connected with plant life , so plants were and still are an important source of his food , medicine and shelter to him , and he resorted to research and study at all times to reveal the secret of this kingdom , relying on the theory of wrong and right , and then what we call Folk taxonomy emerged , and thus the importance of identifying and diagnosing plant species and describing them to benefit from them in all scientific fields (Brahimi , 2003), the Crusader family Cruciferae is also called The Mustard Mustard family or the cabbage family of large plant families that are important medically and economically , widely spread in the world and formerly called the cruciferous family and its name is due to the cruciform shape of the cruciform resulting from the presence of four petals and four sepals in the leaves of the perianth leaves , and one of the important and prominent diagnostic qualities of this family is the number of stamens and their lengths , the pistil an ovary divided by a false septum pseudobulbar , the fruit is either a narrow round silicate mustard or a narrow round siliqui mustard, containing seeds that are in two chambers 2 chambers due to the presence of a false septum pseudobulbar, each side of which is surrounded by a valve shutter of the fruit shutters and other attributes (glimn-Lacy and kaufan, 2006).

The chemical study of plants is concerned with the study of chemical compounds of great

interest and importance , and these compounds are by-products of metabolic processes inside the plant such as phenolic compounds Phenolic Compounds , vitamins Vitamins , terpenes Terpenoids, flavonoids flavonoids and other compounds (Ozkan and et al, 2016), the chemical classification of Chemotaxonomy is based on the diagnosis of the presence of active chemicals in plant organs, whether root, stem or leaves (Rajab and et al, 2014).

Materials and methods

1. Specimens Collection plant and Identification

In the chemical study, he relied on dry plant leaves deposited in the Iraqi national herbarium (BAG) to estimate the phenolic content of the six species under study belonging to the genus Lepidium .the leaves were used after being washed and cleaned well, then dried at room temperature, then ground and placed in dark Vials to ensure that they were not exposed to sunlight .

2. Phenols extraction procedure

Phenolic compounds were diagnosed from the powder of the leaves of the plants under study and dried at room temperature , 1 g of the dried powder of the species under study was taken and extracted using 60 ml of the organic solvent hexane this compound removes oils , fats , waxes and others , the remaining substances were extracted using 50 ml of methanol , then alcohol was evaporated to obtain 3 mL of the high-performance (HPLC), this technique works well to diagnose phenols by acid methanol (John, 1995).

3. Separation condition

Phenols were separated from the mixture by the reverse phase and using a Type C-18 separation column with a diameter of (150 x 4.6 mm) with a volume of minutes with a diameter of 5 micrometers , the mobile phase mobile phase represents 0.7% of acetic acid acetic acid in acetonitrile acetonitrile and water in a ratio of : (1:1) by volume , flow rate flow rate 2 ml per minute at a temperature of 30 degrees Celsius , and through a UV detector UV A detector with a wavelength of 280 Nm detected phenols and the standard concentration was 50 micrometers / ml.

The phenolic content in the species under study was estimated using the following equation:

Compound concentration = the area of the compound bundles in the model/ the area of the standard Compound X the standard concentration X the number of dilutions

The percentage is measured according to the formula :

Percentage = partial concentration / total concentration X 100%, according to (Wollenwebe and et al, 2002)

4. Devices used in the diagnosis of phenols

1. Dual pump model HPLC-10A Shimadzu, japan
2. Reodyne 7125 injection device with an injection ring with a volume of 25 microliters
3. UV reader for wavelength 280Nm

Table 1: shows phenolic compounds in standard analysis, time of detention, area and concentration

Compounds	Concentration	Area	Retention time
QURCETIN	6.8196	3742	3.037
FERULIC ACID	3.2935	2298	5.281
SINAPIC ACID	6.1372	3047	7.016
P-COMARIC ACID	15.8924	6650	9.482
CAFFEIC ACID	3.6473	2674	10.831
CHLOROGENIC ACID	9.0507	4789	11.651
CINNAMIC ACID	6.2981	3095	14.973
EPICATECHUIC ACID	15.4128	6501	16.026
CATECHUIC ACID	3.5145	2383	17.297
KAEMPFEROL	9.0375	4676	18.742
PROTOCHATECHUIC ACID	21.1072	8816	19.553

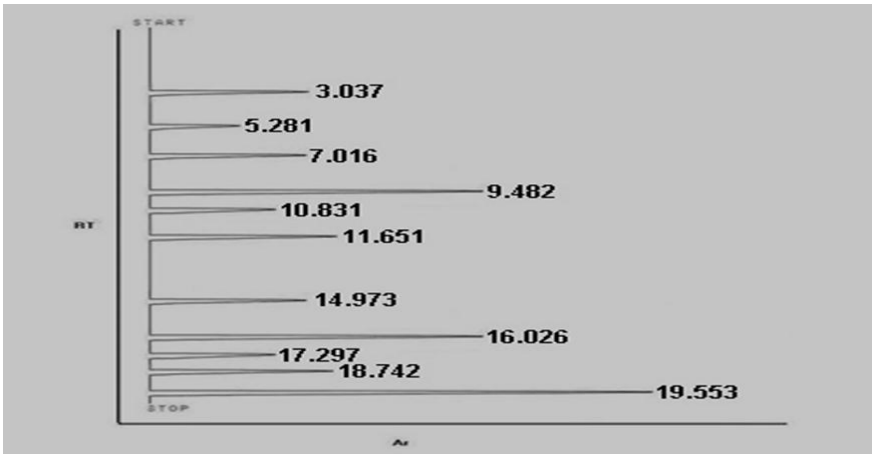

Figure 1: Standard analysis of phenols, time of detention Rt and area Ar

Results and discussion

The current study showed a clear variation in the quality of phenolic compounds, their concentrations and percentages diagnosed using the HPLC high-performance liquid phase chromatography technique, as some phenolic compounds were diagnosed in the leaves of six species of the genus Lepidium, as 11 phenolic compounds were diagnosed in the studied species, as in Table 2 .

The current study , according to the diagnosis of alkaloid compounds in the studied species , showed the presence of 5 compounds Ferulic acid , Sinapic acid , P-Comaric acid, Caffic acid, Kaempferol in all the studied species, namely L.sativum , L.ruderale , L.latifolium , L.persicum , L.perfoliatum , L.aucheri, recorded the highest concentration of the compound Ferulic acid in type L.persicum reached 160.729 mcg / ml with a percentage of 26.5%, while the lowest concentration was in type L.perfoliatum reached 28.046 mcg / ml with a percentage of 4.6% , while the compound Sinapic acid had the highest concentration in type L.aucheri reached 85.050 mcg / ml with a percentage of 22% and the lowest concentration recorded in type L.sativum was 41.04 mcg / ml with a percentage of 10.6%, while the highest concentration of the compound P-Comaric acid was in type L.ruderale reached 53.721 mcg / ml with a percentage of 19.1%, while the lowest concentration was in type L.latifolium reached 23.834 mcg / ml with a percentage of 8.5% , and the highest concentration of Caffic acid compound was recorded in type L.latifolium

reached 119.97 micrograms / ML with a percentage of 25.4%, and its lowest concentration was in type L.perfoliatum reached 26.196 mcg / ml with a percentage of 5.6% , while the compound Kaempferol had the highest concentration in type L.persicum reached 69.835 mcg / ml with a percentage of 24.9% and the lowest concentration was in type L.sativum amounted to 23.42 mcg /ml with a percentage of 8.3%

The current study recorded the presence of the compounds Chlorogenic acid and Epicatechoic acid in five species of the genus Lepidium, namely L.sativum and L.ruderale and L.latifolium and L.persicum and L.perfoliatum this compound did not appear in type L.aucheri, recorded the highest concentration of the compound Chlorogenic acid in type L.ruderale reached 54.552 mcg / ml with a percentage of 25.5%, the lowest concentration was in type L.persicum reached 22.457 mcg / ml with a percentage of 10.5% , while the highest concentration of Epicatechoic acid compound was in type L.persicum reached 55.291 mcg / ml with a percentage of 30.3%, the lowest concentration recorded in type L.perfoliatum was 15.666 mcg / ml with a percentage of 8.5%

The current study showed the presence of 4 phenolic compounds Curcetin, cinnamon acid, Catechoic acid and Protochatechoic acid were diagnosed in four of the studied species, namely L.sativum and L.ruderale and L.latifolium and L.persicum and this compound did not show the two types L.perfoliatum and L.aucheri, where the highest concentration of the compound Curcetin was recorded in type L.sativum amounted to 83.311 mcg / ml with a percentage of 27.8%, the lowest concentration was in type L.persicum reached 67.223 mcg / ml with a percentage of 22.4%, while the compound cinnamon acid recorded the highest concentration in type L.latifolium amounted to 75.282 mcg / ml with a percentage of 28.9%, the lowest concentration was in type L.persicum reached 38.707 mcg / ml with a percentage of 14.8% , while the catechoic acid compound had the highest concentration in type L.latifolium reached 170.814 mcg / ml with a percentage of 34.2%, the lowest concentration was in type L.sativum reached 105.119 mcg / ml with a percentage of 21% , and the protochatechuic acid compound recorded its highest concentration in type L.persicum reached 33.96 mcg / ml with a percentage of 33.3%, the lowest concentration was in type L.sativum reached 17.75 mcg/ml with a percentage of 17.4%, as shown in Table 2

Compounds		**Species**					
		L.aucheri	**L.perfoliatum**	**L.persicum**	**L.latifolium**	**L.ruderale**	**L.sativum**
Qurcetin	**Focus**	**—**	**—**	**67.223**	**72.033**	**77.47**	**83.311**
	The ratio	**—**	**—**	**22.4**	**24**	**25.8**	**27.8**
FERULIC ACID	**Focus**	**54.155**	**28.046**	**160.792**	**131.244**	**118.603**	**113.098**
	The ratio	**8.9**	**4.6**	**26.5**	**21.6**	**19.5**	**18.6**
SINAPIC ACID	**Focus**	**85.050**	**55.513**	**70.110**	**83.442**	**50.44**	**41.04**
	The ratio	**22**	**14.4**	**18.19**	**21.6**	**13**	**10.6**
P-COMARIC ACID	**Focus**	**53.368**	**44.488**	**51.172**	**23.834**	**53.721**	**53.676**
	The ratio	**19**	**15.8**	**18.2**	**8.5**	**19.1**	**19.1**

CAFFEIC ACID	**Focus**	**86.611**	**25.196**	**65.837**	**119.97**	**84.442**	**84.648**
	The ratio	**18.5**	**5.6**	**14**	**25.4**	**18**	**18**
CHLOROGENIC ACID	**Focus**	**—**	**39.162**	**22.457**	**45.186**	**54.552**	**52.64**
	The ratio	**—**	**18.3**	**10.5**	**21.1**	**25.5**	**24.5**
CINNAMIC ACID	**Focus**	**—**	**—**	**38.707**	**75.282**	**71.05**	**75.121**
	The ratio	**—**	**—**	**14.8**	**28.9**	**27.3**	**28.8**
EPICATECHUIC ACID	**Focus**	**—**	**15.666**	**55.291**	**44.87**	**35.063**	**31.32**
	The ratio	**—**	**8.5**	**30.3**	**24.6**	**19.2**	**17.2**
CATECHUIC ACID	**Focus**	**—**	**—**	**105.329**	**170.841**	**118.506**	**105.119**
	The ratio	**—**	**—**	**21**	**34.2**	**23.7**	**21**
KAEMPFEROL	**Focus**	**60.361**	**52.127**	**69.835**	**40.579**	**33.875**	**23.42**
	The ratio	**12.5**	**18.6**	**24.9**	**14.4**	**12**	**8.3**
PROTOCHATECHUIC ACID	**Focus**	**—**	**—**	**33.96**	**27.994**	**22.169**	**17.75**
	The ratio	**—**	**—**	**33.3**	**27.4**	**21.7**	**17.4**

The current study showed that type L.sativum contained the highest concentration of Ferulic acid compound at 113.098 mcg/ml with a percentage of 18.6%, while the lowest concentration was in Protochatechoic acid compound at 17.75 mcg / ml with a percentage of 17.4%, as shown in Table 2 and Figure 2

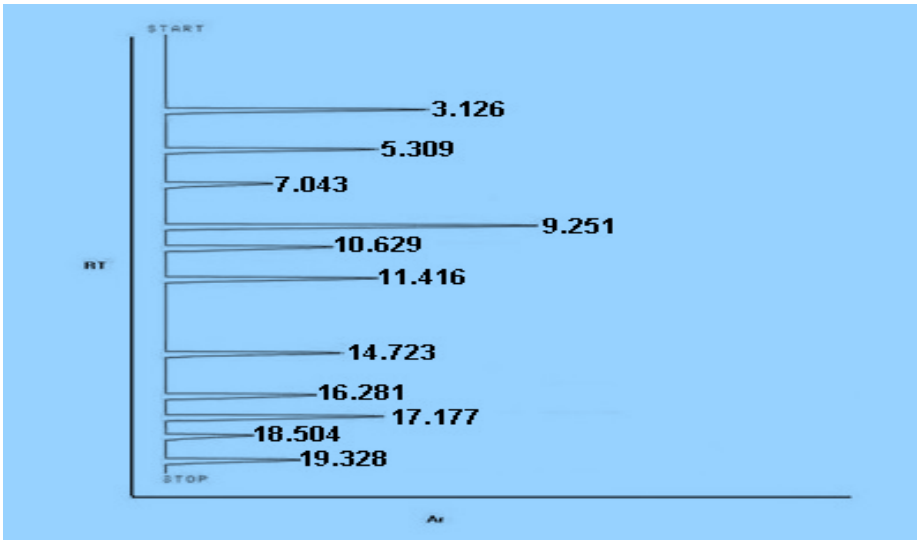

Figure 2: phenols diagnosed in type L.sativum, time of detention Rt and Space Ar

As for the type L.ruderale recorded the highest concentration of the compound Ferulic acid at 118.603 mcg/ml with a percentage of 19.5%, while the lowest concentration was for the compound Protochatechoic acid at 22.169 mcg / ml with a percentage of 21.7%, as shown in Table 2 and Figure 3

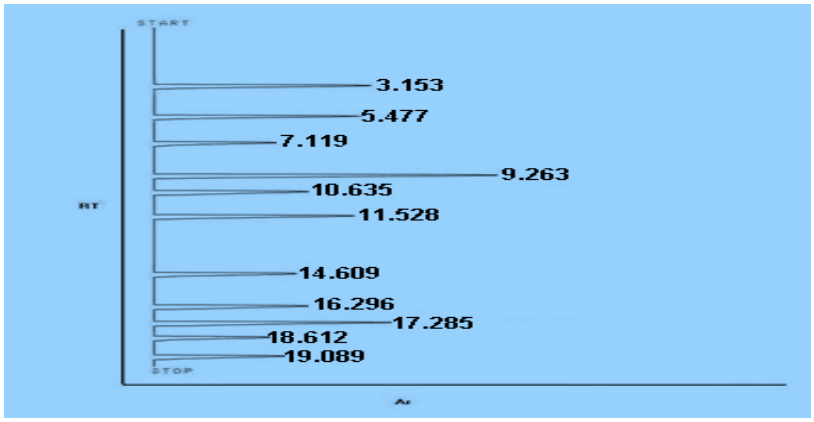

Figure 3: phenols diagnosed in type L.ruderale, time of detention Rt and Space Ar

The type l was recorded.latifolium had the highest concentration in the compound Catechoic acid at 170.814 mcg/ml with a percentage of 34.2%, while the lowest concentration was for the compound P-comaric acid at 23.834 mcg / ml with a percentage of 8.5%, as shown in Table 2 and Figure 4.

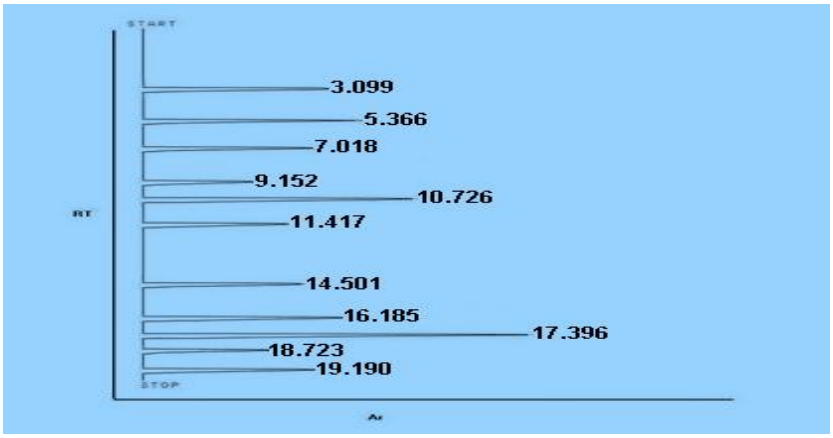

Figure 4: phenols diagnosed in type L.latifolium, time of Rt and Ar

As for the type L.persicum recorded the highest concentration of the compound Ferulic acid at 160.792 mcg/ml with a percentage of 26.5%, while the lowest concentration was in the compound Chlorogenic acid at 22.457 mcg / ml with a percentage of 10.5%, as in Table 2 and Figure 5

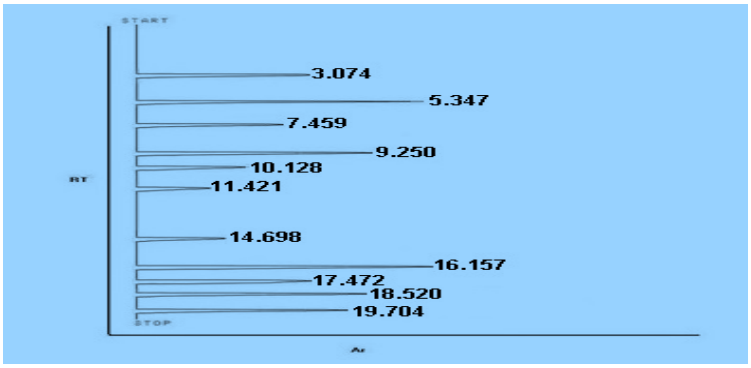

Figure 5: phenols diagnosed in type L.persicum, time of Rt and Ar

While the type l was recorded L.perfoliatum the highest concentration of the compound Sinapic acid reached 55.513 mcg/ml with a percentage of 14.4%, while the lowest concentration was for the compound Epicatechoic acid at 15.666 mcg/ml with a percentage of 8.5%, as in Table 2 Figure 6

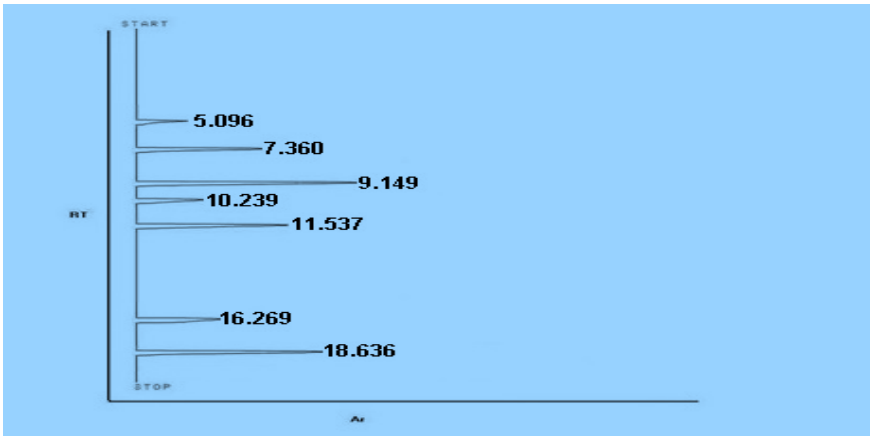

Figure 6: phenols diagnosed in type L.perfoliatum, time of Rt and Ar

The current study showed that type L.aucheri recorded the highest concentration in the compound Caffeic acid at 86.611 mcg/ml with a percentage of 18.5%, while the lowest concentration appeared in the compound P-comaric acid at 53.368 mcg / ml with a percentage of 19%, as shown in Table 2 and Figure 7

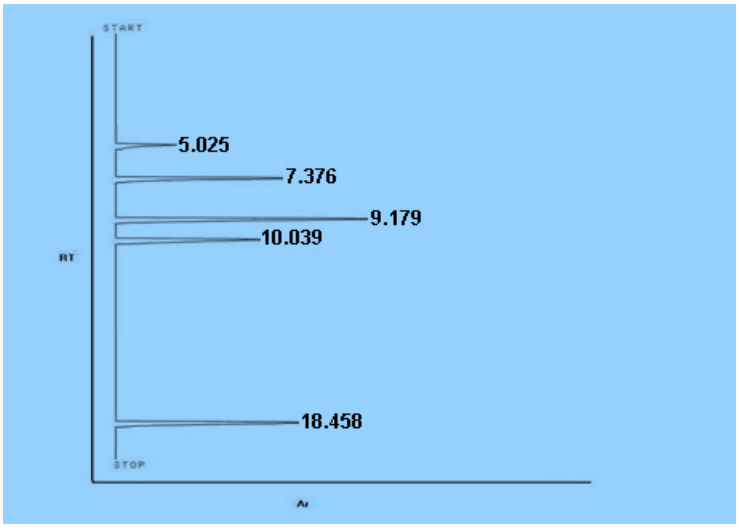

Figure 7: phenols diagnosed in type L.aucheri, time of Rt and Ar

Discussions

The current chemical study showed heterogeneity of the six L species.sativum and L.ruderale and L.latifolium and L.persicum and L.perfoliatum and L.aucheri is in the characteristic chemical compounds since the species contains L.sativum and L.ruderale and L.latifolium and L.persicum is based on 11 chemical compounds , namely Curcetin, Ferulic acid, Sinapic acid, P-Comaric acid, Caffeic acid, CHlorogenic acid, cinnamon acid, Epicatechoic acid, Catechoic acid , Kaempferol and Protochatechoic acid, because these species are the most widespread and the most environmentally adapted, some of them are found in environments harsh desert and

mountainous, as for type L.perfoliatum recorded 7 chemical compounds, while type l containedaucheri has a minimum number of chemical compounds, with only 5 compounds recorded, the presence of these heterogeneous compounds in species and concentrations helps in isolating species from each other , as well as in diagnosing and isolating superficially similar species that are difficult to separate (al-Aboudi, 2017)

The participation of species of the same genus in the same chemical compounds can give an indication of the existence of a common evolutionary Association in terms of chemical properties, and the presence of common compounds in the species of the genus indicates the unity of the genus and the validity of belonging of its species to it as well as the validity of belonging (Muhammad , 2015)

When comparing the results of the current study with the results of some researchers on the same genus and some on the same species, we find there is a variation in some species and concentrations, including the study of Al-Taie and et al (2018) through their study on phenolic compounds of some cabbage varieties and type L.aucheri noted the presence of 9 phenolic compounds , namely chlorogenic acid, cinnamon acid, ferulic acid, genetic acid, kaempferol, p-coumaric acid, protocatechoic acid, sinapic acid and vanillic acid, since type l containedaucheri has the highest concentration of four compounds , including cinnamon acid, genetic acid, p-coumaric acid and sinapic acid, and this is different from what was stated in my current study of the same species, since it does not contain the compounds cinnamon acid and genetic acid, the study of El-salam and et al (2019) showed on the chemical properties of L Seed Powder.sativum the presence of 21 phenolic compounds , namely Gallic, Pyrogallol, Aminobenzoic, Protocatechoic, Catechein, Chlorogenic, Catechol, Caffiene, benzoic, Caffeic, Vanillic, p-Coumaric, Ferulic, Iso - ferulic, Ellagic, Benzoic, Cinnamic and coumarin, salicylic, α - coumaric and methoxy cinnamon in different concentrations and proportions, and this indicates the importance of the seeds of this species from a medical point of view because they contain this number of chemical compounds compared to the leaves of this species, as there are 11 phenolic compounds similar to what was found in the seeds except for the compounds Curcetin, synapic acid, Epicatechoic acid and kaempferol, as for the study of Ali and et al (2021) about phenolic acids for type l shoots.latifolium during different growing weeks, there was a difference in concentrations for the same species, as well as for the same place, but with a different collection time, it contained L-type buds.latifolium contains 8 phenolic compounds, namely Gallic Acid, Chlorogenic Acid, benzoic Acid, Syringic Acid, Vanillic Acid, Caffeic Acid, P-Caumaric Acid and Ferulic Acid, five of which were found in my current study of leaves of the same species

The differences in the types and concentrations of phenolic compounds in the studied species are due to the genus Lepidium L. This is due to several reasons, including the difference in the places of collection of samples, the L species was collected.sativum and L.ruderale and L.latifolium and L.persicum is from the environments of central Iraq, so it is characterized by resistance to difficult environmental conditions from high temperatures and lack of rain, and also the time of collecting samples has a big role in the difference in concentrations of phenolic compounds between species, as well as the nature of breeding, which may be rocky or low in water, as well as environmental stresses and environmental harshness, all these reasons are factors influencing the increase in the concentration and numbers of chemical compounds that have a protective and defensive role for plants (Kerdar and et al, 2018)

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