



A comparative study of the phenolic compounds of some Brassicaceae taxa by High-performance liquid chromatography (HPLC) technique

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Abstract

This study was conducted to determine the phenolic compounds that may be utilized as chemotaxonomic study to identify some phenolic compounds concentration that are useful for pharmaceutical purposes from 5 taxa belongs to the Brassicaceae family which are; *Cardaria draba* (L.) Desv., *Lepidium aucheri* Boiss., *Sisymbrium irio* L., *Sisymbrium officinale* (L.) Scop., and *Sisymbrium septulatum* DC. Leaves powder of these taxa were collected and analyzed by High-performance liquid chromatography (HPLC). The results identified 9 phenolic compounds, chlorogenic acid, cinnamic acid, ferulic acid, genistic acid, kaempferol, p-coumaric acid, protocatechuic acid, sinapic acid, and vanillic acid. The results showed that the genus *Lepidium aucheri* had the highest concentration of 4 phenolic compounds, cinnamic acid, genistic acid, p-coumaric acid, and sinapic acid with concentration of (37.2, 80.79, 62.91, and 37.10 µg/ ml) respectively. On the other hand, the genus *Cardaria draba* showed the lowest concentration of 3 phenolic compounds, cinnamic acid, p-coumaric acid, and protocatechuic acid, and doesn't have 2 phenolic compounds, genistic acid and kaempferol. This variation in the phenolic concentrations might be due to the genotype and plant growth stage differences. The results of this study provide a source for bioactive compounds to future studies.

1. Introduction

Brassica crops contain significant amounts of bioactive compounds practically taxa with large amount of secondary metabolites like carotenoids, different alkaloids, phenolic compounds, and volatile oils. The family Brassicaceae includes more than of 355 accepted genera and approximately 3,500 species. The same taxa has uses depending on forms or types. Phenolic compounds is general term to indicate a numerous compounds (over 8,200) vest distributed in the plants. Phenolic compounds are resulted in plants as secondary metabolites in a reaction catalyzing the coded enzyme [1]. In general, they are classified according to the number and structure of carbon molecular such as flavonoids [1].

Phenolic acids have many significances in plant kingdom, such as share in the natural protective system of plant contrasts many of microorganisms and they considers as a hormone controllers. Moreover, they are having an effective role in

promoting the plant health [2]. in addition, from a chemical point of view, the analyzed data that resulted from chemotaxonomy study provide reliable proof to other aspects. Such as information obtained from cellular, morphological, and anatomical studies can provide a solid foundation for a taxonomic decision, It also can be a good tool to solving morphological problems. The chemical data has been possible to be identified and can be used as a classifying tool to isolate different taxa and categories.

Many studies have reported the determination of phenolic acids by thin-layer chromatography (TLC), and the use of high-performance liquid chromatography (HPLC) is usually used in the identification of many compounds from many families and taxa [3,4] and in different taxa from the family Brassicaceae [5,6], or the isolation of the phenolic compounds structure by using HPLC [7].

To the best of our information, no HPLC method has been reported for determination of phenolic compounds from Brassicaceae's taxa that cultivated in Diyala city, Iraq.

In this paper we review and determination the quantity of phenolic compounds in leaves of 5 taxa belong to Brassicaceae family as chemotaxonomy's marker of isolated genera and to identify the concentration of some phenolic compounds that are useful for pharmaceutical purposes as this can provide beneficial sources for future studies and identified the significance of some bioactive compounds.

2. Material and Methods

2.1 Plants samples

Fresh leaves of 5 genera belong to the Brassicaceae family, *Cardaria draba* (L.) Desv., *Lepidium aucheri* Boiss., *Sisymbrium irio* L., *Sisymbrium officinale* (L.) Scop., and *Sisymbrium septulatum* DC were collected in February 2016. These plants were grown in different regions of Diyala province in the middle of Iraq. The leaves were dried and ground into powder for chemical analysis. This study was conducted at the laboratory of the ministry of science and technology.

2.2 Phenolic Compounds Extraction and Analysis

Five mg of the leaves powder were dissolved in 20 ml methanol HPLC grade, and then were put at ultrasonication (Branson sonifier, USA) with 25 min at 25 °C and 60 % cycles. then centrifuged for 15 min at 7.500 rpm. The clear mixture was collected and was treated by charcoal to remove the pigments under evaporator (Buchi Rotavapor Re-type). Vortexes the samples in 1.0 ml HPLC grade methanol, and then the mixture was passed in 2.5 µg filters and for further analysis samples stored at 4°C. Subsequently, 20µg of each sample injected into HPLC system according to the optimum separation condition. All the used chemicals were from analytical grade, trifluoroacetic acid, and phenolic acids standards were purchased from Sigma - Aldrich (Steinheim, Germany). The compounds were spilt on Fast Liquid Chromatographic (FLC) column under the optimum condition, column: pheomenex C-18, 3µg particle size (100x4.6 mm I.D) column. Mobile phase: linear gradient of solvent A was 0.1 % trifluoroacetic acid (TFA) in deionized water; solvent B was acetonitrile gradient programmed from 0% B – 100% B for 10 min. The flow rate has been 1.3 ml/min followed by the UV at 280 nm. The sequences of eluted material of the standard were at Table 1. The calculation of the concentration of phenolic compounds in the sample $\mu\text{g/ml} = \text{area of sample} / \text{area of standard} \times \text{cons. Of standard} \times \text{dilution factor}$. The separation occurred on liquid chromatography Shimadzu 10AV- LC equipped with binary delivery pump model LC-10A Shimadzu. The eluted peaks were monitored by UV/Vis 10A-SPD spectrophotometer [8].

Table 1. Phenolic compounds that used in study as standard

Seq	Phenolic compound (20µm)	Retention time (minute)	Area µ volt
1.	Chlorogenic acid	4.45	20820
2.	Cinnamic acid	1.18	556553
3.	Ferulic acid	6.34	19962
4.	Genstic acid	2.03	19736
5.	Kaempferol	8.21	26787
6.	p- coumaric acid	5.28	24976
7.	Protocatechuic acid	9.59	38105
8.	Sinapic acid	5.28	24245
9.	Vanillic acid	2.95	29228

3. Results and Discussion

The identification of 9 compounds belonging to the phenolic family in the taxa under study is shown in Table 1. and Figure 1.

In this study, the result showed that there are significant differences in the compounds levels in the genera under study. The genus *Lepidium aucheri* recorded the highest concentration of 4 types of phenolic compounds, while the species *Cardaria draba* showed the lowest concentration of 3 types of phenolic compounds, while it was lacking 2 types of phenolic compounds, Genstic acid and Kaempferol. On the other hand, chlorogenic acid characterized by highest abundance (122.21µg/ml) in species *Sisymbrium irio*, with the lowest concentration of (35.18 µg/ml) in *Sisymbrium officinale*.

Interestingly, cinnamic acid in these genera showed the highest concentration in *Lepidium aucheri* of (37.20 µg/ml), but less concentration in *Cardaria draba* of (2.24 µg/ml). The results of this work agree with [5] where they reported that the hydroxycinnamoylquinic acids was the most abundant in leafy *Brassica* species. However, previous studies showed that kaempferol and sinapic acid have been shown high important in *Brassica* taxa. Ferulic acid reported highest concentration of (78.74 µg/ml) in *Sisymbrium irio*, but in *Sisymbrium septulatum* has showed the lowest concentration of (6.26 µg/ml). This outcome was in disagreement with [9] where in their study, they found only ferulic and sinapic acids appeared in incorporeal quantities in roots. This might be due to evolutionary relationships among the different *Brassicaceae* taxa.

On the other hand, genstic acid has been found in *Lepidium aucheri* with a concentration of (80.79 µg/ml), while this acid was not found in species *Cardaria draba*. However, the kaempferol concentration in *Sisymbrium septulatum* was of (49.80µg/ml), while this compound was not found in *Cardaria draba*. The authors [10] reported that kaempferol and sinapic acid were the greatest detected phenolic that was determinate by HPLC analysis. This attributes to differences genotype in the taxa of this study, but on the other hand, it is considered evolutionary evidence with species *Cardaria draba* due to it different content and varied concentration.

Apart from that, p- coumaric acid was indicating to have the highest concentration in *Lepidium aucheri* of (62.91 μ g/ml) while it showed less concentration in species *Cardaria draba* with (21.95 μ g/ml). And in the same line, sinapic acid showed the highest level in the *Lepidium aucheri* with a concentration of (37.1 μ g/ml), but in species *Sisymbrium officinale* it reached (8.18 μ g/ml). This result was in agremnt with [10] where they reported that sinapic acid was one of the major phenolics acids that were isolated from the leaves of Brassicaceae taxa using HPLC technique.

Moreover, the protocatechuic acid which present in the taxa under this study reached its highest concentration in *Sisymbrium septulatum* with a concentration of (21.40 μ g/ml), but in the *Cardaria draba* it was only (6 μ g/ml). In addition, the vanillic acid in *Sisymbrium irio* showed the highest concentration of (45.2 μ g/ml) while it showed less concentration in *Sisymbrium septulatum* with a concentration of (6.63 μ g/ml).

These findings indicate that the presence of these compounds is considered as an evolutionary phenomenon of importance aspect [11]. The result of

this study showed that all taxa under the study share particular compound which supports the existence of evolutionary inter-genera of this family, in terms of their chemical properties. This indicates the possibility of isolation of taxa taxonomically from each other, according to the presence of phenolic compounds and their concentrations. This also confirms the possibility of using such materials in the insulation taxa, especially when there are interlocking in the morphological characters, as it will be more difficult to be separated.

These phenolic compounds provide an evidence for the important role of helping or supporting the entrusting phenotypic study [3]. Moreover the determination of the concentration of these phenolic compounds can help to choose the best taxa that can be used in pharmaceuticals. Finally, it is worthy to mention that phenolic compounds concentration depends on many factors such as analysis method and environmental factors [12]. Therefore, this study deals mainly with the phenolic profile of *Brassicaceae* crops, as well as the differences in concentrations of these compounds.

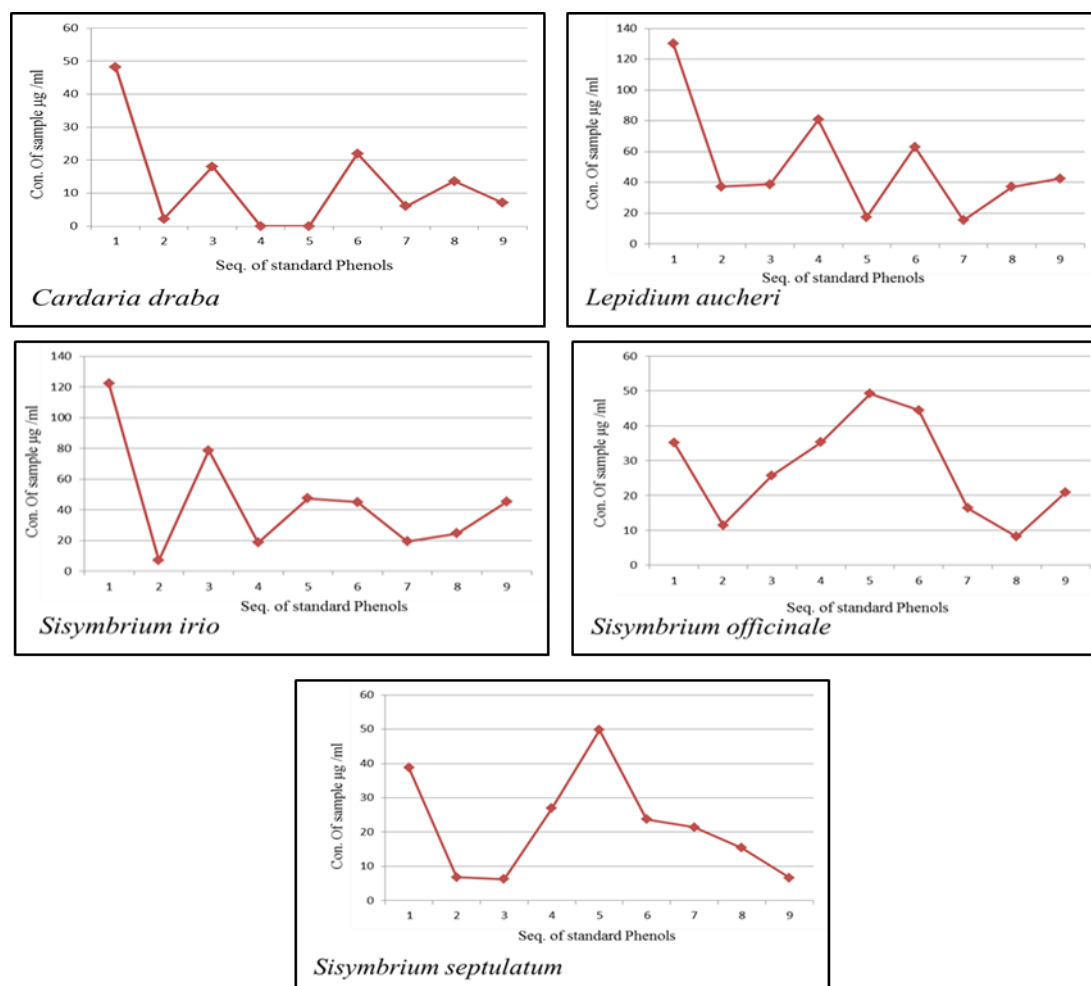


Figure 1. Phenols in the some Brassicaceae taxa, Chlorogenic acid1.Cinnamic acid, 2.Ferulic acid, 3.Genstic acid, 4.Kaempferol, 5.p- coumaric acid, 6.Protocatechuic acid, 7.Sinapic acid, 8. Protocatechuic acid, 9.Vanillic acid.

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دراسة مقارنة للمركبات الفينولية لبعض أنواع العائلة الصليبية بواسطة تقنية كروماتوجرافيا السائلة عالية الأداء

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الملخص

أجريت هذه الدراسة لتحديد المركبات الفينولية التي يمكن استخدامها كدراسة كيميائية مع تحديد بعض تركيز المركبات الفينولية التي هي مفيدة للأغراض الصيدلانية من 5 أصناف تنتمي إلى العائلة الصليبية وهي:

Cardaria draba (L.) Desv. , *Lepidum aucheri* Boiss. , *Sisymbrium irio* L. , *Sisymbrium officinale* (L.) Scop., and *Sisymbrium septulatum* DC.

تم جمع مسحوق الأوراق من هذه الأصناف وتحليلها باستخدام الكروماتوجرافيا السائلة عالية الأداء. حددت النتائج 9 مركبات فينولية وهي حمض الكلوروجينيك وحمض سيناميك وحمض الفيروليك وحمض الجينستيك وكامفيرول وحمض الكوماريك وحمض البروتاكوتشيك وحمض السنايبك وحمض الفانيليك.

أظهرت النتائج أن جنس *Lepidum aucheri* كان أعلى تركيز ل 4 مركبات فينولية وهي حمض سيناميك وحمض جينستيك وحمض كوماريك وحمض سنابي مع تركيز (37.2 و 80.79 و 62.91 و 37.10 µg / مل) على التوالي. من ناحية أخرى، أظهر جنس *Cardaria draba* أقل تركيز من 3 مركبات الفينولية، حمض سيناميك، p- حمض الكوماريك، وحمض بروتاكتشيك، وكان يفتقر إلى 2 المركبات الفينولية، وحمض الجينستيك و كامفيرول. هذا الاختلاف في تركيزات الفينول قد يكون راجعاً إلى النمط الجيني والاختلاف في مرحلة النمو. توفر نتائج هذه الدراسة مصدراً للمركبات النشطة بيولوجياً للدراسات المستقبلية.

الكلمات المفتاحية: العائلة الصليبية في العراق، كروماتوجرافيا السائلة عالية الأداء، المركبات الفينولية، التصنيف الكيميائي.