Isolation and Identification of Phenolic Compounds from the Plant Residues of *Hordeum* sp. and *Brassica* sp. by Using High Performance Liquid Chromatography (HPLC)

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**ABSTRACT**

The current study included qualitative diagnosis and quantitative estimation of some active compounds in two types of weeds, namely wild mustard *Hordeum* sp. and wild barley *Brassica* sp. The results showed that these plants contained several chemical compounds that were diagnosed as phenolic acids. Wild mustard contained three chemical compounds, namely: Galic acid, Resorsenol, and Vanillin. While wild barley contained four compounds, namely: Galic acid, Resorsenol, Vanillin, and benzoic acid. The results of quantitative estimation also showed variation in the concentration of these compounds in the extracts of these weed residues.

**Keywords:** weed, phenolic compounds, wild mustard, Wild barley.
INTRODUCTION

Weed plants are one of the most dangerous agricultural pests that affect the agricultural production in the world, causing great losses for agriculture in Iraq, in productivity and economic returns (Verma et al., 2002). In Iraq, there are many weeds that compete with agricultural crops in food, water and air... and thus reduce its productivity and quality (Al-Chalabi et al., 2010) and the percentage of losses caused by weeds in economic crops is between 20-85% (Al-Ali, 1980). The successful emergence of weed in any ecosystem is attributed to the fact that the plants of the natural weed are characterized by having a strong root group with active vegetative growth and resistance to environmental conditions (high and low temperatures, drought, low fertility of the land and various soils) and produce (Saeed, 2010). In addition, the seeds of some types of weeds contain more than one embryo, and each number and the seeds of many weeds go into a period of dormancy and thus retain its vitality for a long time (Abu Bakr, 2003). One of the most important weeds that depletes soil moisture is wild mustard. the weed of wild barley causes many problems in the permaculture, and that is its similarity with cereal crops, which makes problems of permaculture, and due to the studies on these two weeds. (Ibrahim et al., 2020) botanical description of the bush plants used in the study 1 - wild barley Brassica sp. the northern part of Iraq is abundant and is considered a broad-leaved weed, which is a member of the Brassicaceae family. multiplying with seeds that number up to 400 seeds per day, Single plant, straight stem and up to 80-100 cm high, flowers cruciform yellow, seeds semi-globular smooth. Black (Al-Ali et al., 2010). 2 - Wild barley Hordeum sp. is a herbaceous annual winter plant, which is a member of the Gramineae family. Seeds, with straight stems branched, leaves simple striped and flowers in spike inflorescences at the end of the stem, multiply by seeds. The number of seeds formed by the plant is 5000 seeds, and when cutting the stem, a red sap appears, which disappears during the flowering period, the seed is small, spear-shaped (Al-Ali et al., 2010).

Extraction of phenolic acids from plant residues of the weed

The phenolic acids were extracted according to the method (Grand et al., 1988). The plant residues were dried for bush cultivation at degree 40°C and then crushed and ground using an electric grinder (to ensure that the phenolic compounds do not volatilize).

Weigh 10 g of each sample, then add to it 100 ml of ethyl alcohol 70%, and put the mixture. The electric vibrator for an hour, then kept in the refrigerator for 24 hours, then put it back on the electric vibrator. for an hour to ensure the release of the compounds, then filter the solution using filter papers (No.1 Whatman). Focus the filter to a volume of 25 ml at 40°C. After that, an acid analysis was carried out by taking (10) ml of the filtrate and adding to it (50 ml of 1N HCl acid) prepared by adding (7.1 ml of acid with 3.8 ml of distilled water) The mixture was placed on a device (Mental) at a temperature of 100°C for half an hour, and a thermal escalation process was conducted for it. Then the solution was cooled and filtered using filter papers (No.1Whatman) to remove sediments, Transfer the filtrate to a funnel. The funnel separating was added to it and 15 ml of ethyl acetate was added and left until the top two layers were formed. Represents (ethyl acetate with phenolic acids), and the bottom contains fats and other materials, the bottom layer was collected to be returned. The process must be repeated again and left for ten minutes until the separation process is completed. Thus, the ethyl acetate Extract contains free phenols that are ready for diagnosis using HPLC technology Harborn, (1973). Six standard compounds obtained from multiple stores were prepared, as 100) mg / 30 ml was dissolve. From ethanol and filtered (Robbert et al., 2000) and became ready standard compounds, then the steps were carried out (20) μl of the previously prepared standard compounds were injected into the HPLC device supplied by Company (SUPELCOSIL TM Coulnum) separation column using (LC2010 HT) Japanese type (Shimadzo) It has dimensions of 6mm x 4 15cm. Also, 20 microliters were injected from each sample of plant extracts. In the HPLC device for the purpose of detecting compounds expected to appear, and the transfer phase (acetonitrile: water) was used 10. 90with a flow speed of 3.1 ml/min, and the
detection of chromatographic responses at a wavelength of 320 nanometers. (Al-Jubouri, 2007). The number of phenolic compounds was estimated using the following law:

\[
\text{Amount of separated substance} = \frac{\text{area under the curve of the sample} \times \text{concentration of the standard compound}}{\text{area under the curve for the standard compound} \times \text{sample volume ml} / \text{sample weight g}} \quad (\text{Al-Losi, 2014})
\]

RESULTS AND DISCUSSION

Diagnostics and quantitative determination of phenolic compounds in plant residues of weeds, wild mustard and barley using HPLC technology. The results of the detection showed the presence of four chemical compounds in the plant residues of the weed (wild mustard and wild barley). In addition to the presence of other vehicles that have not been diagnosed because the time of their detention does not match the time of detention of the standard vehicles available the wild mustard extract contained compounds (Vanilin, Resorsenol, acid Galic), while the wild barley extract contained (Benzoic, Vanilin, Resorsenol, Galic acid) as shown in (Table 1), showing the values of the retention time (Rt) for the compounds diagnosed in plant extracts for planting with this technology, it seems clear from the table that the highest percentage of the number of diagnosed compounds was in (wild barley) residues.

Table 1: Phenolic compounds and retention time values diagnosed by HPLC technology for ethanol extract of weed residues (wild mustard and wild barley)

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Vehicles Retention time (min (Rt.))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>the sample standard</td>
</tr>
<tr>
<td>1 Benzoic</td>
<td>2.293</td>
</tr>
<tr>
<td>2 Galic acid</td>
<td>1.898</td>
</tr>
<tr>
<td>3 Resorsenol</td>
<td>1.82</td>
</tr>
<tr>
<td>4 Vanillin</td>
<td>1.98</td>
</tr>
</tbody>
</table>

Figs. (1-2) represent the absorption peaks of phenolic compounds in the residues of the studied wild mustard and wild barley and it is clear from these curves that there are several compounds in each curve that were diagnosed by HPLC technology. Extract of wild mustard bush residues. The results of separation by HPLC technology indicated the presence of a number of absorption peaks. The most prominent of them was the absorbance peak of the compound (Gallic acid) with a retention time of (1.877), which is close to the retention time for the standard sample of (Gallic acid) compound (1.898) with an area under the curve (42.737), (as the absorbance peak was separated for the compound (Resorsenol) with a retention time of (1.73) (which is close to the retention time of the standard sample of the compound (Resorsenol) (1.82) and an area under the curve (41.96) And the absorption peak of the compound (Vanillin) with a retention time of 2.02 which is Approach to the standard sample retention time of (vanillin) compound with area under the curve (22.42).
Fig. 1: Absorption peaks of phenolic compounds separated by HPLC technology in wild mustard residues

As for the samples of wild barley residue extract, the results of separation using HPLC technology indicated the presence of a number of peaks absorption, the most prominent of which was the absorbance peak of (Gallic acid with a retention time of (1.878), which is close to the time of the retention of the standard sample of (Gallic acid) with an area under the curve (58.47 as the absorption peak was separated) for a compound (Vanillin) with a retention time of (2.00), which is close to the retention time of the standard sample for a compound (Vanillin) with an area Under the curve (15.06), the absorption peak of (benzoic) with a retention time (2.106), which is close to the time of benzoic as well as the standard sample retention absorbance for a compound (benzoic) with an area under the curve (17.95). For the compound (Resorsenol) with a retention time of 1.71 and an area of (3.28), which is close to the retention time of the standard compound (Resorsenol).
Quantitative determination of separated phenolic compounds from weed residues (wild mustards and wild barley)

The concentration of compounds was calculated and quantified based on HPLC by comparing the area under the curve to a technology analysis for the standard material with the area under the curve for the weed residue samples, and the results of (Table 2) indicated that there are significant differences in the concentration of the separated chemical compounds, as it was found that the highest concentration of (Gallic acid) was observed in wild barley residues amounted to (0.413) µg/g, while the compound (Resorsenol) was given the highest concentration The residues of the wild mustard weed reached (0.04 (µg/g), while the concentration of (Vanilin) was (0.067) µg/g in wild mustard weed residues.
Table 2: Estimation of the number of phenolic acids (µg/gm) diagnosed using HPLC technology for the extract

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Barley wild</th>
<th>Mustard wild</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Benzoic</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>2 Galic acid</td>
<td>0.413</td>
<td>0.302</td>
</tr>
<tr>
<td>3 Resorsenol</td>
<td>0.014</td>
<td>0.040</td>
</tr>
<tr>
<td>4 Vanillin</td>
<td>0.045</td>
<td>0.067</td>
</tr>
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</table>

In our study, a number of compounds were separated and diagnosed from the wastes of the weed used in the study (wild mustard and wild barley) (according to the available from standard compounds) studies indicate that plant parts contain a large number of compounds that secondary metabolites are produced by plants in different ways, but there are many factors that influence their nature and their quantity due to the different environmental conditions and biotic and abiotic factors that affect the formation of compounds and their concentrations. in plants, as well as plant growth stage and plant tissue type (Manjula and Mythili, 2012). These results agree with the study of Al-taee (2017), where the results indicated that wild mustard extract, wild barley extract contained the compounds (Hydroquinone, Coumarin, Salicylic acid, Catechol) in varying quantities and concentrations.

REFERENCES


فصل وتشخيص وتحديد المستوى الكمي للمركبات الفينولية في المخلفات النباتية لدغمي الخردل البري والشعير البري *Brassica sp.* والشعير البري *Hordeum sp.*

**High performance Liquid Chromatography (HPLC)**

المختصر

تضمنت الدراسة الحالية التشخيص النوعي والتركيز الكمي لبعض المركبات الفعالة في نوعين من الأدغال وهي الخردل البري *Brassica sp.* والشعير البري *Hordeum sp.* أظهرت النتائج احتواء هذه النباتات على عدة مركبات كيميائية شخصت هذه المركبات كيميائية شخصت على

Gallic acid, Resorsenol, Vanillin, يحتوي الخردل البري على ثلاثة مركبات كيميائية وهي في حين احتوى الشعير البري على أربع مركبات وهي:

benzoic acid, Vanillin, Resorsenol, Galic acid

كما أظهرت نتائج التقدير الكمي تباينا في تركيز تلك المركبات في مستخلصات مخلفات تلك الأدغال.

الكليات النافعة: الأدغال، المركبات الفينولية الخردل البري، الشعير البري.