Rafidain Journal of Science
https://rsci.mosuljournals.com

Vol. 31, No. 4, pp.8-14, 2022

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

Rawnaq A. Ibrahim
Department of Biology/ College of Education for Girls/ University of Mosul
Janan A. Saeed

Department of Biology/ College of Science/ University of Mosul

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Article information

Received: 13/5/2022
Accepted: 31/7/2022

DOI: 10.33899/rjs.2022.176068

corresponding author:
Rawnaq A. Ibrahim
rawnakaltaee@uomosul.edu.iq

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, Vanillin While wild barley contained four compounds, namely: Galic acid, Resorsenol, Vanillin 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 effect on 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 Brassiceceae 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:

Amount of separated substance = area under the curve of the sample x concentration of the standard compound/ area under the curve for the standard compound x sample volume ml / sample weight g (Al-Losi, 2014).

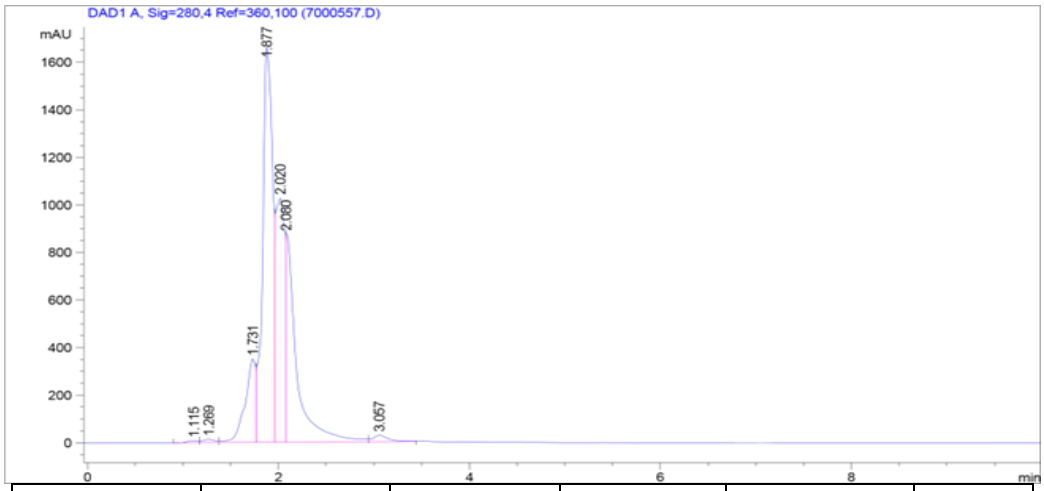
RESULTS AND DISCUSSION

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)

	Phenolic compounds	**'Vehicles Retention time (min (Rt .)**		
		the sample standard	**barley wild**	**mustard wild**
1	Benzoic	2.293	2.106 1	____
2	Galic acid	1.898	1.878	1.877
3	Resorsenol	1.82	1.71	1.73
4	Vanillin	1.98	2.000	2.02

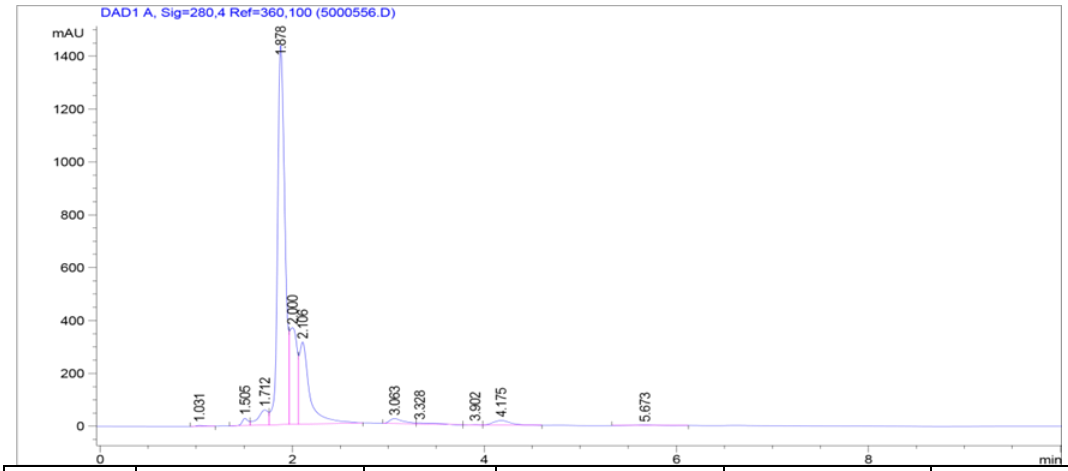
Figs. (1-2) represent the absorption peaks of phenolic compounds in the residues of the studied wild mustard and wild barley and it is clear from these curves that there are several compounds in each curve that were diagnosed by HPLC technology. Extract of wild mustard bush residues. The results of separation 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).



# Peak	Ret .Time min	Width min	Area Mau*s	uHeight ma	% Area
1	BV1.115	0.1333	78.48103	9.22250	0.2725
2	VV1.269	0.1274	128.76689	14.52384	0.4471
3	VV1.731	0.1097	2701.48486	351.24078	9.3790
4	VV1.877	0.1123	1.23097 e4	1664.31543	42.7370
5	VV2.020	0.0854	6458.68359	1026.48865	22.4233
6	VV 2.080	0.1294	6802.33154	876.02344	23.6164
7	VB3.057	0.1663	323.93314	27.73188	1.1246
Total			2.88034e4	3969.54652	

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).



# Peak	Ret .Time min	Width min	Area Mau*s	Height mau	% Area
1	1.031	0.0884	20.78129	3.44580	0.1586
2	VV 1.505	0.0650	121.34462	27.78094	0.9261
3	VV 1.712	0.1091	430.50504	57.66444	3.2857
4	VV 1.878	0.0820	7661.78857	1441.29272	58.4770
5	VV 2.000	0.0829	1974.33020	365.93774	15.0687
6	VB 2.106	0.1081	2352.2302	311.53745	17.9529
7	BV 3.063	0.1475	193.08609	18.85449	1.4737
8	VV 3.328	0.2107	76.12660	4.65732	0.5810
9	VV 3.902	0.1151	9.30920	1.24630	0.0711
10	VB 4.175	0.1951	222.04951	17.60656	1.6947
11	BB 5.673 11	0.2938	40.66904	2.10135	0.3104
Total			e41.31022	2252.12511	

Fig. 2: Absorption peaks of phenolic compounds separated by HPLC technology in wild barley residues

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

	Phenolic compounds	Barley wild	Mustard wild
1	Benzoic	0.072	_____
2	Galic acid	0.413	0.302
3	Resorsenol	0.014	0.040
4	Vanillin	0.045	0.067

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

Abu Bakr, S.N. (2003). "Plant Pests and Diseases". Part 1. Food and Agriculture Organization (FAO) of United Nations, Agricultural Program for Security Council Resolution 9865, Erbil, Iraq.

Al-Ali, A.S.; Istafan, Z.A.; Awad, H.I. (2010). "Agricultural Pest Control Manual Ministry of Agriculture". Republic of Iraq.

Al-Ali, A. (1980). "Agricultural Pest Control Manual". The Ministry of Agriculture and Agrarian Reform. The Public Authority for the Protection of Crops, Prevention Research Department, Public Authority for Agricultural Education and Guidance Press, Baghdad, Republic of Iraq

Al-Chalabi, F.T.; Al-Bahadli, A.A.J. (2010). Competitiveness of some sorghum cultivars for an accompanying weed. *Iraqi J. Agricult. Sci*., **41**(3), 63-78.

Al-Jubouri, S.H.A. (2007). A physicochemical study of extracts of pentosans and their related components. Some locally grown cultivars of wheat and their relation to the hardiness of the bean. PHD thesis, College of Agricultural and Forests, University of Mosul.

Al-Losi, O.W.J. (2014). The role of the growth regulator jasmonic acid in causing a change in the level of metabolism Secondary and salinity resistance in callus cultures of *rosea Vinca*. L Master Thesis, College of Science, Tikrit University, Iraq.

Al-taee, R.A. (2017). Allelopathic effect of three weed residues on growth and nodulation of tow legume species. Master Thesis, College of Science, Mosul University, Iraq.

Grand. A.V.; Ondergen, R.; Pousset, J. (1988). Anti-infection phytotherapies of tree-savannah., Senegal (West Africa), Antimicrobial activity of 33 species. *J. Ethnopharmaco*., **22**, 25-31.

Hrborn, J.B. (1973). "Phytochemical method". London. Chapman and Hall, Ltd. pp. 49-188

Ibrahim, R.A.; Saeed, J.A. (2020). allelopathic effect of three weed residues on growth and nodulation of *Visia faba* and *lens culinaris* medic. *Rafidian J. Sci*., **29**(1), 1-10.

Manjula, R.; Mythili, T. (2012). Improved phytochemical production using biotic and abiotic elicitors in Marsilea quadrifoli Int. *J. Curr. Sci.*, 98-101.

Robbert, J.R.; Smith, F.A. (2000). The limits of sodium calcium interaction in plant growth. *Aust. J. Plant Physiol*., **27,** 709-715.

Saeed, J.A. (2010). Allelopathic interaction of prostrate Spurage *Euphorbia prostrate* L. with three species of Ornamental plant. *Rafidian J. Sci*., **21(**3), 24-37.

Verma, R.; Rao, P.B.; Rawat, Y.S. (2002). Studies on allelopathic influence of five weeds on the germination of ten varieties of *Brassica*. *Indian J. Envi. Sci*., **6**,113-118.

فصل وتشخيص وتحديد المستوى الكمي للمركبات الفينولية في المخلفات النباتية لدغلي الخردل البري والشعير البري.*Brassica* sp *Hordeum* sp. باستخدام تقانة High performance Liquid Chromatography (HPLC)

الملخص

تضمنت الدراسة الحالية التشخيص النوعي والتقدير الكمي لبعض المركبات الفعالة في نوعين من الادغال وهي الخردل البري *Brassica* sp والشعير البري *Hordeum* sp. . واظهرت النتائج احتواء هذه النباتات على عدة مركبات كيميائية شخصت على انها احماض فينولية وقد احتوى الخردل البري على ثلاثة مركبات كيميايية وهي : Galic acid , Resorsenol, Vanillin

في حين احتوى الشعير البري على أربع مركبات وهي:

benzoic acid , Vanillin, Resorsenol, Galic acid

كما اظهرت نتائج التقدير الكمي تباينا في تركيز تلك المركبات في مستخلصات مخلفات تلك الادغال.

الكلمات الدالة: الادغال، المركبات الفينولية الخردل البري، الشعير البري.