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Comparative Studies among some Cultivated species and their Wild Relatives in Egypt Using Phytochemical Screening, Nutritive value, Antioxidant Activity and GC-MS Analysis

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ABSTRACT

Climate change is a major threat to agricultural sustainability and productivity around the world. For this, modern breeding strategies have focused on improving crop qualities through crop wild relatives (CWRs). Increased genetic diversity and enhanced agricultural resilience through the utilization of crop wild relatives (CWRs), which are closely related to cultivated crops, offer a sustainable approach for crop development amidst ongoing climatic concerns. In order to provide effectively data in agronomic, nutritional, and breeding efficiency traits for crop advances, this study aims to provide some phytochemical proprieties and biochemical attributes for some cultivated plants and their wild relatives belonging to three families; Apiaceae, Asteraceae and Brassicaceae. The main and secondary metabolites of the chosen domesticated plants were differed from those of their wild counterparts, according to the results of the phytochemical screening. Wild *Lactuca serriola* (Asteraceae) has the highest nutrient density (457.21 K cal/ 100 g) and *Apium leptophyllum* has the lowest (381.94 Kcal/ 100 g). Comparing IC50 values, *Daucus carota* has the lowest (0.052 mg/ml) and *Lactuca serriola* has the greatest (0.457 mg/ml) antioxidant activity. Identification of the phytoconstituents and GC-MS analysis is one of the best and accurate techniques to nature of active principles in medicinal plants.

Keywords: Antioxidant activity; Climate change; Crop wild relatives; Gas chromatography-Mass Spectroscopy; Nutritive value; Phytochemical analysis.

INTRODUCTION

Climate change is a pervasive and growing global threat to biodiversity and ecosystems (Díaz et al., 2019). Climate change affects individual species and the way they interact with other organisms and their habitats, which alters the structure and function of ecosystems and the goods and services that natural systems provide to society (Díaz et al., 2019). Understanding the direction and magnitude of ecological responses allows human communities to better anticipate these changes and adapt as necessary. However, developing appropriate adaptation plans can be challenging because species, populations, and even entire ecosystems may respond to climatic changes in different ways (Weiskopf et al, 2020).

The role of plant breeding in adapting crops to climate changes that affect food production in developing countries is recognized as extremely important and urgent, alongside other agronomic, socio-economic and policy adaptation pathways. To enhance plant breeders' capacity to respond to climate challenges, it is acknowledged that they need to be able to access and use as much genetic diversity as they can get (Galluzzi *et al*., 2020). Genetic diversity can be used in crop improvement to make crop varieties with desirable traits like higher drought and heat tolerance and more efficient use of inputs (Prohens *et al*. 2017). Crop wild relatives have most of the genetic diversity in crop genepools. This is so that farmers and consumers can get the quality and quantity of crops they want (Mousavi-Derazmahalleh *et al.*, 2018; Allen *et al.*, 2019).

Crop wild relatives (CWR) are plants that grow in the wild but are genetically related to crops. They are thought to have the most valuable traits that can be used in plant breeding and crop improvement to make sure that food and nutrients will be available in the future (Herden et al, 2020; Kioukis et al, 2020). CWR are very important in plant breeding and crop improvement because they have important traits that make agricultural production hardy and high-yielding (Kishii, 2019). The main benefit of CWR is that it makes it easy to put genes into crop varieties to help them deal with biotic and abiotic stresses caused by climate change (Dempewolf et al. 2014). Due to their bioactive and antioxidant properties, which are caused by the presence of chemically effective compounds, medicinal plants play an important role in both the food system and the treatment of diseases (Agbor et al., 2011). These chemical compounds are powerful groups of compounds that plants make naturally and that may be good or bad for health (Silva *et al.*, 2017). They also split into two groups: the primary metabolites and the secondary metabolites (Balandrin *et al*., 1985).

Primary metabolites are very important to plants and take part in the primary metabolic processes that build and keep plant cells (Briskin, 2000). Primary metabolites are made up of nucleic acids, proteins, carbohydrates, and lipids. They can be found in all plants (Erd and Kliebenstein, 2020). On the other hand, secondary metabolites, which are also called phytochemicals and come from primary metabolites, are called biologically active compounds because they have a pharmacological effect on the human body (Meksi, and Moussa, 2017; Ivanovi, *et al.*, 2020). Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. are some of the most important phytochemicals that come from different parts of plants (Sheel *et al*., 2014, Shi *et al.*, 2022).

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These chemicals that plants make are not only used by the plants to protect themselves from biotic and abiotic stresses, but they have also been turned into medicines that people can use to treat different diseases (Hidalgo, et al., 2018). These compounds also help treat diabetes, reduce inflammation, fight free radicals, and protect nerve cells (Gulcin *et al.,* 2011).

Plants possess the remarkable ability to synthesize antioxidants as secondary metabolites, which play a vital role in scavenging reactive oxygen species (ROS) or free radicals across various biological systems (Abuajah et al., 2015). The accumulation of excessive ROS and free radicals within living organisms has been strongly linked to the development of chronic and degenerative diseases, including age-related pathologies, cancer, cardiovascular disorders, immune-deficiency syndrome, diabetes, among others. These detrimental effects occur due to the damaging influence exerted by ROS and free radicals on essential biomolecules such as carbohydrates, proteins, lipids, and nucleic acids - processes that contribute to the onset of these diseases (Talaz *et al.,* 2009). However, when present in limited quantities within biological systems, antioxidants effectively counteract this oxidative damage by mitigating the harmful impact of ROS and free radicals (Zehiroglu and Sarikaya, 2019).

Gas chromatography–mass spectrometry (GC–MS) is widely recognized as one of the most reliable and accurate techniques for identifying various chemical compounds (Konappa *et al.,* 2020). This analytical method plays a pivotal role in the detection and characterization of diverse compounds, including alkaloids, nitrogenous compounds, alcohols, long-chain hydrocarbons, organic acids, esters, steroids, and amino acids present in medicinal plants (Razack *et al.,* 2015). GC-MS combines two instrumental components: gas chromatography (GC), which separates mixture components based on their differential movement between the mobile phase and stationary phase; and mass spectrometry (MS), which measures the mass-to-charge ratio of electrically charged species. As a result, GC-MS enables efficient separation and identification of target substances with high precision and sensitivity (Pawar *et al.,* 2021).

Apiaceae is one of the largest plant families. It has over 3,780 species in 434 genera that grow all over the world (Ahmad *et al.,* 2017). In addition to, plants in the Apiaceae family are grown for their taste, smell, and health benefits. There are glands that store essential oils (EOs) in every part of Apiaceae plants (Sousa et al., 2021). EO, which gives each species its own flavor, is a very important part of a plant's defense against bacteria, viruses, fungi, insects, herbivores, and other pests. It also helps pollinators find the plant (Christensen and Brandt, 2006). There are about 25,000 species and 1600 genera in the Asteraceae family, which is also called the sunflower family. This family is very important because it contains a lot of biologically active substances, such as essential oils and polyphenolic compounds like lignans, saponins, polyphenolic compounds, phenolic acids, sterols, and polysaccharides (Rolnik and Beata, 2020; Shamso *et al.,* 2021). They are also good sources of Na, K, Ca, and Mg, as well as vitamins A, B, C, and D.

One of the largest angiosperm families, Brassicaceae (Cruciferae), consists of 12–15 tribes, 338–360 genera, and about 3709 species that are found all over the world (Muhlhausen *et al*., 2013). Brassicaceae grows in both arid and semi-arid areas, and it is very sensitive to both biotic stresses, like those caused by bacteria, viruses, and fungi, and abiotic stresses, like cold, heat, salt, and drought (Kayum *et al*., 2016). This family of plants has a wide range of economic uses, including as food, feed, medicine, research model plants, high-yield crops, and decorative plants. This Brassicaceae plant is a great source of vitamins A, B1, B2, B6, C, E, K, and minerals Mg, Fe, and Ca (Shankar *et al*., 2019). This investigation aimed to study the comparative analysis among five cultivated species and their five wild relatives from three families (Apiaceae, Asteraceae & Brassicaceae) based on their phytochemical components, nutritive value, antioxidant activity and their chemical constituents using GC Mass analysis.

MATERIALS AND METHODS

Collection of plant materials

Viable seeds of cultivated species were obtained from National Gene Bank, ministry of Agriculture and Land Reclamation, Egypt. Then, these seeds were planted in Mansoura university field and after approximately 3 months of growth, shoot system were harvested as shown in Figure (1) while wild relatives were collected from natural habitats from Egypt as shown in Table (1). The wild relatives were identified by prof. Dr. Ibrahim A. Mashaly Professor of Plant Ecology and Flora, Botany Department, Faculty of Science, Mansoura University, and herbarium specimens were deposited in herbarium of Botany Department, Faculty of Science, Mansoura University, Egypt. Identification and nomenclature were according to Tackholm (1974).

Sample preparation for analysis

Sample preparation for analysis involved immediate transportation of collected plants to the laboratory. The shoot system of each species was precisely cleaned and thoroughly washed with distilled water multiple times. Subsequently, the plant materials were dried at 55 to 60°C in a forced air oven for 24 hours to achieve optimal moisture reduction. Following drying, the plant samples were finely ground and transferred into sample bottles for subsequent analysis.

Determination of chemical constituents of selected plant species

Determination of some primary compounds

Determination of total lipid

The determination of total lipid content involved extracting ten grams of plant sample powder using a Soxhlet apparatus. The extraction process utilized petroleum ether (boiling point 60-80°C) as the solvent, and it was carried out for duration of 16 hours.

Following extraction, each extract was dried over anhydrous Na_2SO_4 and evaporated to remove any remaining solvent. The resulting residue was further dried at 80 °C for ten minutes, allowed to cool, weighed, and expressed as a percentage of lipid content according to the AOAC method (1990).

Estimation of total protein

The estimation of total protein content involved determining the nitrogen content in each sample using the micro-Kjeldahl method (Chibnall et al., 1943). The protein content was then estimated using the following equation, as specified by the AOAC method (1990).

% protein content =N X 6.26

Where, *N* is the nitrogen content and 6.25 is the protein conversion factor.

Estimation of total carbohydrates

The total carbohydrate content was estimated according to Hedge and Hofreiter (1962). About 0.2 g of powder for each sample was hydrolyzed with 5 cm^3 of HCl (2.5 N) for 3 hours in boiling water bath and then cooled at room temperature. The mixture was then neutralized with Na_2CO_3 until the ebullition disappeared; the volume was raised to 100 cm^3 and then centrifuged, the supernatant was collected. About 4 cm^3 of anthrone reagent was added to 1 cm^3 of supernatant; the mixture heated in a boiling water bath for 8 min and cooled rapidly then read the green to dark green color at 630 nm using spectrophotometer.

Nutritive value

The nutritive value for each studied plant was calculated following the method reported by Indrayan *et al.* (2005), using the equation:

Nutritive value = 4(protein content) + 9(lipid content) + 4(carbohydrate content)

Determination of some secondary metabolites

Total flavonoid contents

Total flavonoid content was measured by a colorimetric estimation using aluminum chloride (Zhu *et al*., 2010) and quercetin used as standard. Total flavornoid content was expressed in microgram quercetin equivalents (QE) per gram dry sample (mg QE/g).

Tannins content

The analysis of tannin contents was conducted using the vanillin-hydrochloride assay method (Burlin-game, 2000; Aberoumand, 2009). The measured values of tannin content in the extracted plant samples were expressed as grams of tannic acid equivalents per 100 grams of dry plant material. The tannin content in the investigated samples was quantified by constructing a standard curve using tannic acid. The equation of the tannic acid standard curve was determined as y = 0.0009x, with an r^2 value of 0.955.

Alkaloids content

Fifty mL of 10% acetic acid in ethanol was added to 1 gm of the sample, covered and allowed to stand for 4 hours at room temperature. Then, sample filtered and was concentrated on a water bath. Concentrated ammonium hydroxide was added on top drop wisely to the extracted mixture until the precipitation process was occurred. The solution was allowed to settle; the precipitate was collected and washed with diluted ammonium hydroxide then was filtered and dried to a constant weight (Harborne, 1973).

Saponins content

Saponins content were determined according to Obadoni and Ochuko, (2001). About 20 g of each sample powder, 20% aqueous ethanol were added and then boiled for 4 hours with continuous stirring. The mixture was filtered and reduced to 40 ml over water bath at about 90°C.The concentrate (1ml) was transferred into separator funnel and 2 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and about 4 ml of n-butanol were added and washed twice with 2 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Antioxidant Activity

The antioxidant activity for all tested plants were estimated using their ethanolic extracts using DPPH (1,1-diphenyl-2-picryl hydrazyl) colorimetric method compared to ascorbic acid as a standard according to (Miliauskas *et al.* (2004) with slight modifications by Lim *et al.,* (2007) as follows: 2ml of 0.15 mM DPPH was added to 1 ml of various plant extracts. A control was prepared by adding 2 ml of DPPH to 1 ml solvent (methanol 50 %). The contents of the tubes were mixed and allowed to stand for 30 min, and measured absorbance at 517 nm. The antioxidant activity was expressed as:

% radical scavenging activity = [1– (A sample/ A control)] x 100

Where, A is absorbance at 517 nm.

IC50 which denotes the amount (mg) of plant in 1 ml solution required to reduce initial concentration of DPPH radicals by 50 % was also calculated. The antioxidant activity of Ascorbic acid was assayed for comparison.

GC/MS analysis

GC/MS analysis of the selected plant extracts was conducted using GC-MS-QP2010 Ultra analysis equipment (Shimadzu Europa, Duisburg, Germany). The temperature of the oven was initially set at 50°C and maintained for 3 minutes. It was then increased at a rate of 8°C per minute until reaching 250°C, where it remained constant for 10 minutes. For GC/MS detection, an electron ionization system with an ioniz-ation energy of 70 eV was utilized. Helium gas (99.999% purity) served as the carrier gas at a consistent flow rate of 1 ml/min., while an injection volume of 2 µl was employed with a split ratio of 10:1. The injector temperature was set to be at 250°C, whereas the ion-source temperature stood at280°C. Subsequently, identification of major individual components took place by employing WILEY and National Institute of Standards and Technology (NIS- T08) libraries based on their relative indices and mass spectra.

Figure (1): The morphology of different plant species used in the study. A, *Apium gravolens* L.; (B). *Apium graveolens* (Pers.) F.Muell; (C), *Daucus carota* L.; (D), *Daucus litoralis* Sm.; (E), *Lactuca sativa* L.; (F), *Lactuca serriola* L.; (G), *Brassica oleracea* L.; (H), *Brassica tournefortii* Gouan; (I), *Raphanus sativus* L. and (J). *Raphanus raphanistrum* L.

Table (1): The scientific name, collection area and GPS of the wild collected taxa from Egypt

Classification of the used plants		Collection Area	Governorate	Location	
				Longitude (E)	Latitude (N)
Family	**Taxa**				
Apiaceae	*Apium leptophyllum* (Pers.) F. Muell.	Mansura University	El-Dakahlia	31° 22' 59.99"	31° 02' 60.00"
	Daucua litoralis Sm.	Gabal ELnargis-Baltim	Kafr EL-Sheik	31° 05' 18.00"	31° 35' 34.19"
Asteraceae	*Lactuca serriola* L.	Mansoura University	El-Dakahlia	° 22' 59.99"	31° 02' 60.00
Brassicaceae	*Brassica tournefortii* Gouan.	Ash-shihabiyah-Baltim	Kafr EL-Sheik	31° 6' 37.584"	31° 35' 47.3244"
	Raphanus raphanistrum L.	Mansoura University	El-Dakahlia	31° 22' 59.99"	31° 02' 60.00"

Statistical Analysis

The results of the whole study are presented in means ±Standard Deviation (±SD). Data were analysed using the statistical package for social sciences (SPSS) 16 and were evaluated by analysis of variance (ANOVA). Duncan's test was used for comparisons among different treatments. Statistical differences were considered significant at the $p \leq 0.05$ level 3. Principal Component Analysis (PCA), Cluster Heat-map and correlation coefficient were used to examine the relationship between the studied taxa and their phytochemical analysis using PAST software (ver. 4, Past Software, University of Oslo, Oslo, Norway).

RESULTS

Preliminary phytochemical screening analysis

Figure (2) presents the results of the preliminary phytochemical screening conducted on the studied taxa, encompassing total carbohydrates, total proteins, and total lipids. Additionally, a comparison of these essential constituents between cultivated species and their corresponding wild relatives is depicted for each species. In the Apiaceae family, the cultivated species *Apium gravolens* has the highest total protein content (19.58%), while the wild species *A. leptophyllum* has the highest total carbohydrate content (56.39%) and total lipid content (14.29%). The cultivated species *Daucus carota* had the highest total carbohydrates (49.046%), while the wild species D. *litoralis* had the highest total protein (18.58%) and total lipid (19.19%).

In the Asteraceae family, *Lactuca sativa* has the highest total protein content (43.6%), while *L. serriola* has the highest total carbohydrate content (38.73%) and total lipid content (26.69%). In Brassicaceae, *Brassica oleraceae* had the highest amount of total carbohydrates (51.528%) and total protein (18.53%), while *B. tournefortii* had the highest amount of total lipid (21.24%). *Raphanus sativus* had the highest total carbohydrate content (52.557%), while *R. raphanistrum* (a wild species) had the highest total protein content (41.78%) and total lipid content (7.19%).

Nutritive value

Nutritive values of selected taxa are shown in Figure (3). In family Apiaceae, the lowest value (381.94 Kcal/100g) is recorded in *A. graveolens* while the highest value (401.96 Kcal/ 100g) is recorded in *A. leptophyllum*. In *Daucus* sp., both *D. carota* and *D. litoralis*) have relatively the same value of nutritive value as 408.83 and 404.96 Kcal/ 100g, respectively. In family Asteraceae, the lowest nutritive value was 401.79 Kcal/ 100g characteristic for *L. sativa* but the highest value was 457.21 Kcal/ 100g) found in *L. serriola.* In Brassicaceae family, *B. oleracea* shows the higher nutritive value (399.11 Kcal/ 100g) than *B. tournefortii* (371.1 Kcal/ 100g), while *R. raphanistrum* has the highest nutritive value (422.02 Kcal/ 100g) than *R. sativa* (400.16 Kcal/ 100g).

Secondary constituents' analysis (phytochemicals analysis

Figure (4) and estimate the total phenols, total flavonoids, total alkaloids, total saponins, and total tannins of some cultivated plants and their wild relatives. In Apiaceae, *Apium gravolens* had the highest levels of total phenols (1.37%), total flavonoids (1.63%), and alkaloids (1.22%) in comparison with *A. leptophyllum* that had the highest levels of saponins 1.171%) and tannins (13.37%). For D. *carota* had the highest levels of total phenols (2.35%), alkaloids (1.057%), saponins (1.77%), and tannins (13.67%), in comparison to *D. litoralis* which had the highest levels

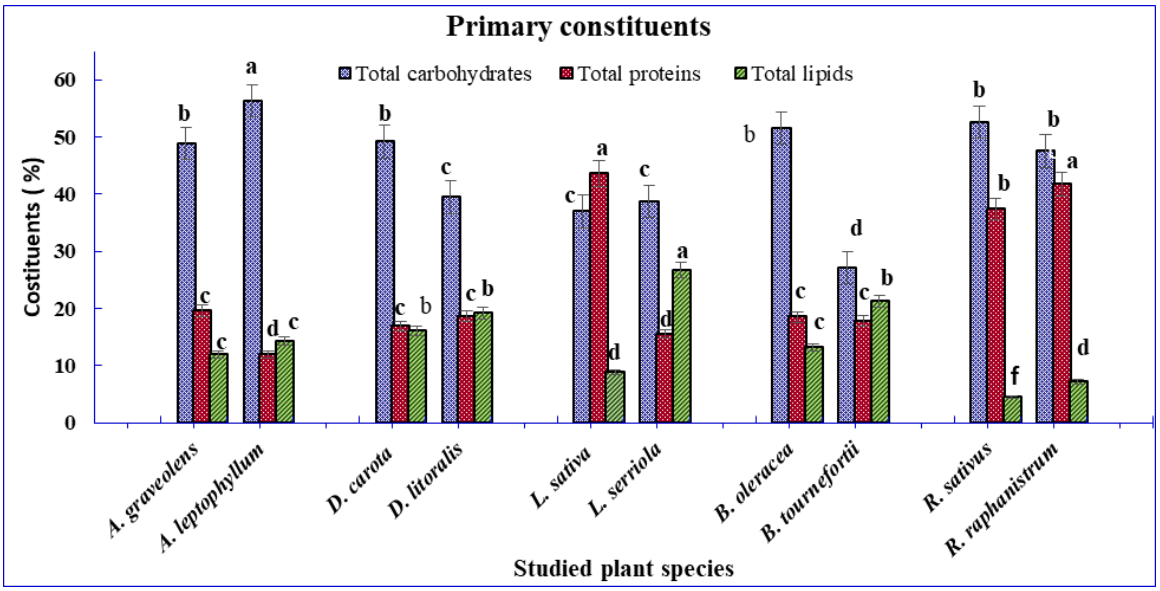

Figure (2): Comparative analysis of primary metabolite percentages between cultivated species and their wild relatives. Columns, per studied genera and their wild types, with different letters are significant different at $p \leq 0.05$ based on Duncan multiple range test

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of total flavonoids (2.54%). Regarding for Family Astraceae, *L. sativa* had the highest levels of total phenols (2.15%), alkaloids (0.586%), saponins (0.465%), and tannins (10.99%), while *L.serriola* only had the highest levels of total flavonoids (1.12%). Finally, *B. oleracea* had the highest alkaloids (1.197%) and saponins (0.044%) in the Brassicaceae family, while *B. tournefortii* had the highest total phe-nols (2.02%), total flavonoids (3.16%), and tannins (34.39%). *R. sativus* had the highest levels of total phenols (2.07%), total flavonoids (1.35%), and alkaloids (1.46%), while *R. raphanistrum* only had the highest levels of saponins (4.702%) and tannins (26.51%).

Antioxidant Activity by DPPH free radical scavenging activity

The antioxidant activity of selected plants is estimated and recorded in Figure (5). In *Apium* sp., the highest radical scavenging activity (IC50) value was 0.3199mg/ml and recorded in *A. graveolens* extract while *A. leptophyllum* shows the lowest value of the IC50 (0.091mg/ml). In *Daucus* sp., the highest IC50 value was 0.061mg/ml in *D. litoralis* extract while *D. carota* shows the lowest value of the IC50 (0.052 mg/ml). In family Asteraceae, the highest IC50 value was 0.457 mg/ml in *L. serriola* extract, while the lowest value of the IC50 was 0.187mg/ml) in *L. sativa*. In Brassicaceae family, according to the IC50 values of *Brassica* sp. methanolic extract, the highest IC50 (0.403 mg/ml) is recorded in *Brassica oleracea* methanolic extract while *Brassica tournefortii* shows the lowest value of the IC50 (0.096mg/ml). In *Raphanus* sp., the highest IC50 (0.639 mg/ml) is recorded in *Raphanus sativus* methanolic extract while *R. raphanistrum* shows the lowest value of the IC50 (0.511 mg/ml).

GC-MS analysis of selected plants

In this study, GC-MS analysis of selected cultivated plants and their wild relatives revealed the presence of several bioactive compounds. On the chromatogram, the peak area (percent) and retention time of chemical compounds were recorded. According to *Apium* species, GC-MS analysis revealed the existence of 10 main peaks in both cultivated *A. graveolens* and its wild related *A. leptophyllum*, as shown in Figure (6). The major chemical constituents (Table 2) of *A. graveolens* were 1-hexyl-2-nitrocyclohexane (33.243%), Ethanol (13.556%), Dodecanoic acid (9.983%), and Butanenitrile, (9.983%), as shown in Table (2), whereas *A. leptophyllum* were 9,17-octadecadinal (61.998%), oxalic acid (8.348%), Ethanol, 2,2'-oxybis-diacetate (7.944%) and L-Valine (6.404%) as in Table (3).

GC-MS analysis for *Daucus* sp. was shown in Table (4) and Table (5). It revealed that presence of 15 major peaks at *D. carota* as in Figure (7A), while *D. litoralis* has only 10 major peaks as in Figure (7B). The major compounds detected in *D. carota* were oxalic acid (22.586%), Acetyl valeryl (22.155%) and Ethanol (20.871271%) while the major chemical constituents for *D. litoralis* were ethanol (30.520597%), propanoic acid (15.435%) and α- pinene (9.22164%).

In *lactuca*, GC-MS result analysis is recorded in Tables (6 and 7). About fifteen major peaks are recorded in *Lactuca* sp. for both *L. sativa* and *L. serriola* as shown in Figures (8A and B). The chemical composition detected in *L. sativa* contains high concentration of were Hexadecanoic acid (37.175%), 3-methyl heptane (20.227%), durenol (9.0073163%) as in Table (6), while the major chemical constituents in *L. serriola* were Hexadecanoic acid (47.199926%), 3-methyl heptane (24.207%) and p-Cymene (16.473%) as Table (7).

In family Brassicaceae, *Brassica* species, GC-MS analysis showed the presence of 16 major peaks for both *B. oleracea* and *B. tournefortii* as shown in Tables (8 and 9) and Figure (9 A and B). The major chemical compounds detected in *B. oleracea* were 3-(methylsulfinyl) propyl (77.018073%), D-Limonene (7.3346144%) and 4-Mercaptobutyl (4.42098%); while

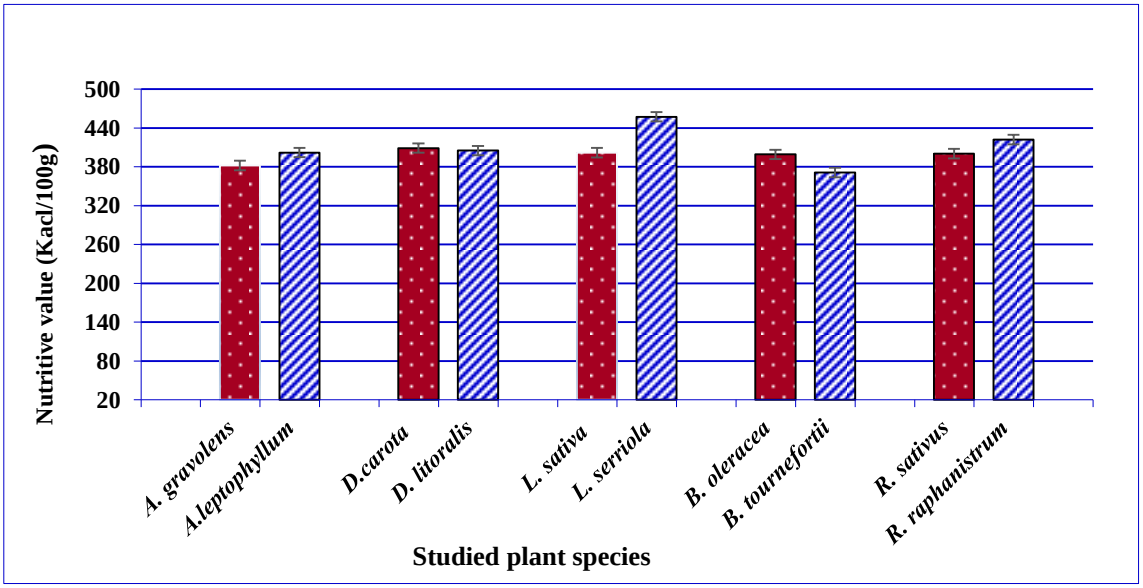

Figure (3): Comparative nutritional composition analysis of cultivated plants and their wild relatives.

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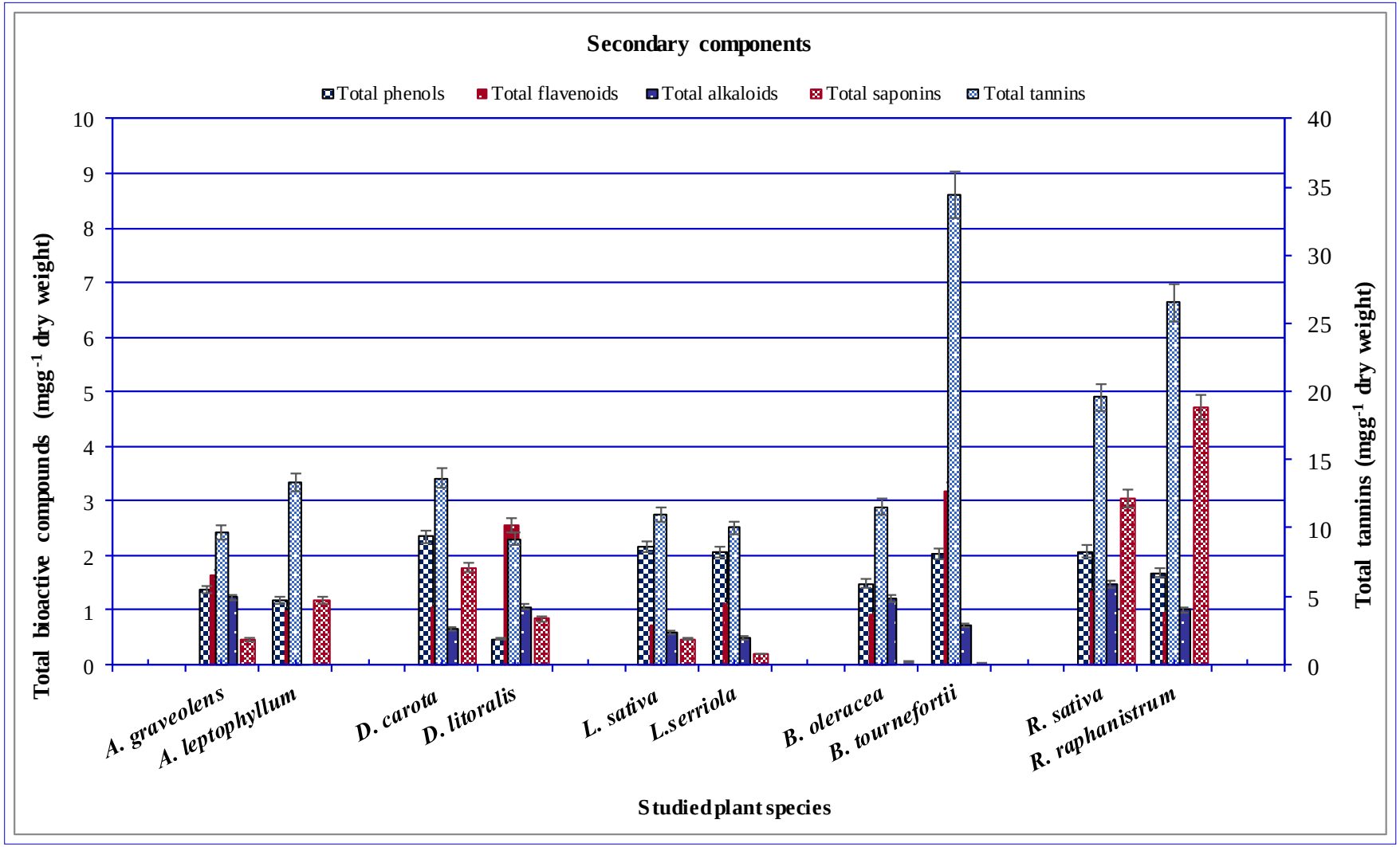

Figure (4): Exploring the bioactive constituents diversity: a comparative analysis between cultivated plants and their wild relatives.

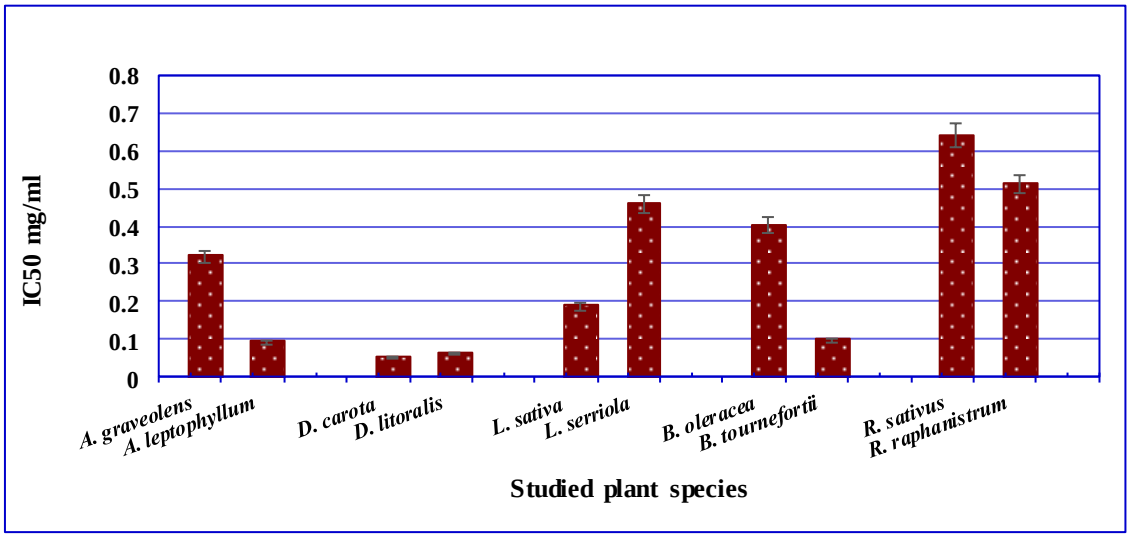

Figure (5): Showing the Comparison of the antioxidant DPPH results expressed as IC50 (mg/ml) values of the selected samples

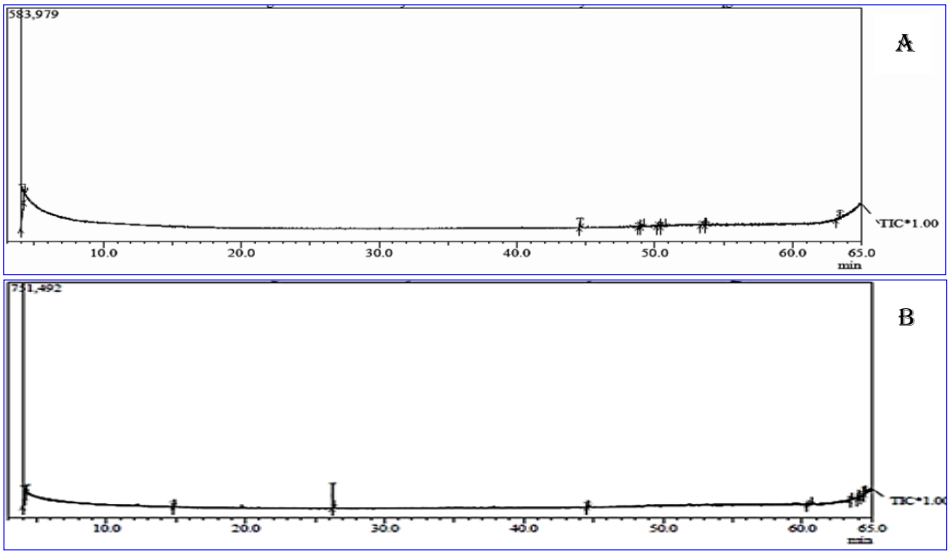

Figure (6): GC–MS chromatogram of volatile components in methanolic extract of the studied plants. A, *Apium graveolens* methanolic extract; B, wild *Apium leptophyllum.*

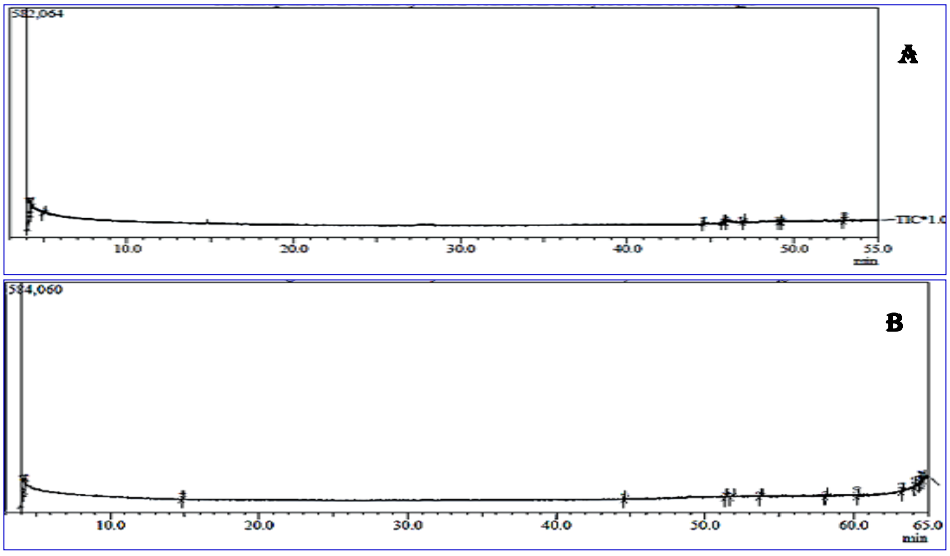

Figure (7): GC–MS chromatogram of volatile components in methanolic extract of the studied plants. A, cultivated *Daucus carota s*; B, *Daucus litoralis.*

Table (2): The relative percentage of main volatile components in cultivated *Apium graveolens* methanolic extract.

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	Methyl Alcohol (solvent)	4.062	1045	-----	RI, MS
2	Ethanol	4.25	1234	13.55612381	RI, MS
3	1-Hexyl-2-nitrocyclohexane	44.59	1763	33.24328119	RI, MS
4	Oxalic acid, diallyl ester	48.84	1834	6.28700361	RI, MS
5	Isobutyl ether	49.07	1921	6.06972857	RI, MS
6	Dodecanoic acid	50.4	1975	9.983487097	RI, MS
7	Butanenitrile	50.4	1977	9.983487097	RI, MS
8	Oxalic acid	53.42	2076	6.74244551	RI, MS
9	Silane	53.76	2079	5.49729242	RI, MS
10	l-Leucine	62.28	2354	5.39701163	RI, MS
11	Others	-----	-----	3.24	RI, MS

Table (3): The relative percentage of main volatile components in wild *Apium leptophyllum* methanolic extract

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	Methyl Alcohol (solvent)	4.104	967	-	RI, MS
2	Ethanol	4.256	982	7.943767274	RI, MS
3	3-Hexanone	14.813	1274	3.227217594	RI, MS
4	9,17-octadecadinal	26.278	1563	61.99817065	RI, MS
5	Oxalic acid	44.577	1672	8.348412241	RI, MS
6	Dodecanoic acid	60.142	1828	2.213119644	RI, MS
7	Ethanesulfonyl chloride	63.716	1892	1.86315643	RI, MS
8	1H-Purine-8-propanoic acid	64.113	1912	2.89614444	RI, MS
9	L-Valine	64.154	1923	6.40373029	RI, MS
10	Glycine	64.532	1957	1.593724523	RI, MS
11	Others	-----	-----	3.5121	RI, MS

Table (4): The relative percentage of main volatile components in cultivated *Daucus carota* methanolic extract.

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	Methyl Alcohol (solvent)	4.149	894	-	RI, MS
2	α-pinene	4.175	902	1.129307	RI, MS
3	Ethanol	4.246	923	20.871271	RI, MS
4	Acetyl valeryl	14.843	1356	22.15501	RI, MS
5	Oxalic acid	44.577	1756	22.58614	RI, MS
6	Acetic anhydride	51.24	1832	1.547569	RI, MS
7	Allyl o-nitrophenyl sulfoxide	51.483	1855	14.49438	RI, MS
8	4-Penten-2-one	53..841	1896	2.158875	RI, MS
9	2-Propenoic acid	58.067	1953	0.608088	RI, MS
10	4-Methyl-2,4-bis(4'-trimethyl silyloxyphenyl)pentene-1	60.315	1981	1.695569	RI, MS
11	Propanoic acid	63.415	2074	2.129918	RI, MS
12	Cyclohexene	64.097	2126	2.303658	RI, MS
13	Succinic acid	64.215	2165	2.982529	RI, MS
14	Pilocarpine	64.459	2187	1.377047	RI, MS
15	1,3-Dioxolane	64.713	2205	1.11322	RI, MS
16	Others	-----	-----	2.85	RI, MS

Table (5): The relative percentage of main volatile components in wild *Daucus litoralis* methanolic extract

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	Ethanol	4.244	794	30.520597	RI, MS
2	α-pinene	4.11	783	9.22164	RI, MS
3	Diethyl azodicarboxylate	4.9	817	11.100623	RI, MS
4	Furan	44.743	1546	5.8221635	RI, MS
5	Diethyl azodicarboxylate	45.816	1581	7.7930904	RI, MS
6	5-Hexen-3-one	45.864	1603	6.5886351	RI, MS
7	2-Butanone	46.971	1692	4.742307	RI, MS
8	3,4-Hexanedione	49.256	1792	3.364168	RI, MS
9	Butyl glyoxylate	49.327	1804	2.174816	RI, MS
10	Propanoic acid	53.027	1973	15.435152	RI, MS
11	others	-----	-----	3.26	RI, MS

Table 6: The relative percentage of main volatile components in cultivated *Lactuca sativa* methanolic extract.

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	3-Methyl heptane	4.049	865	20.22785	RI, MS
2	3,4-Epoxy-2-hexanone	4.164	869	2.3110466	RI, MS
3	1-Decene	4.253	892	1.9595586	RI, MS
4	β-Pinene	4.297	899	1.4740858	RI, MS
5	p-Cymene	4.993	916	8.2012189	RI, MS
6	Hexadecanoic acid	6.196	937	37.175272	RI, MS
7	Limonene	6.824	980	1.2342235	RI, MS
8	Linalool	7.228	1021	6.467955	RI, MS
9	2,4-Dimethyl-pentanol	12.207	1235	3.5917633	RI, MS
10	Thymol	12.866	1282	2.6804134	RI, MS
11	Durenol	13.01	1338	9.0007316	RI, MS
12	4- teroineol	13.176	1384	0.688648	RI, MS
13	α- teroineol	40.389	1764	0.488939	RI, MS
14	Euganol	44.556	1794	1.6532534	RI, MS
15	Camphene	61.074	2073	1.2750529	RI, MS
16	others	-----	-----	1.57	RI, MS

Table (7): The relative percentage of main volatile components in wild *Lactuca serriola* methanolic extract.

ID	Name of the compound	Retention Time	Retention Index	Peak area (%)	Identification
1	3-Methyl heptane	4.107	794	24.207362	RI, MS
2	3,4-Epoxy-2-exanone	4.145	804	3.5603263	RI, MS
3	p-Cymene	4.207	845	16.472971	RI, MS
4	β-Pinene	4.715	982	2.436273	RI, MS
5	Hexadecanoic acid	6.197	1165	47.199926	RI, MS
6	Limonene	6.903	1195	0.8673321	RI, MS
7	Linalool	7.512	1234	0.5281931	RI, MS
8	2,4-Dimethyl-pentanol	7.678	1276	0.133728	RI, MS
9	Thymol	40.416	1967	0.190871	RI, MS
10	3-Octanone	44.564	2034	2.2332291	RI, MS
11	Euganol	45.481	2150	0.117541	RI, MS
12	α- teroineol	49.992	2227	0.185588	RI, MS
13	4- teroineol	50.294	2381	0.133855	RI, MS
14	n-Heneicosane	51.682	2396	0.08208	RI, MS
15	Camphene	64.085	2531	0.065343	RI, MS
16	Others	-----	-----	1.591	RI, MS

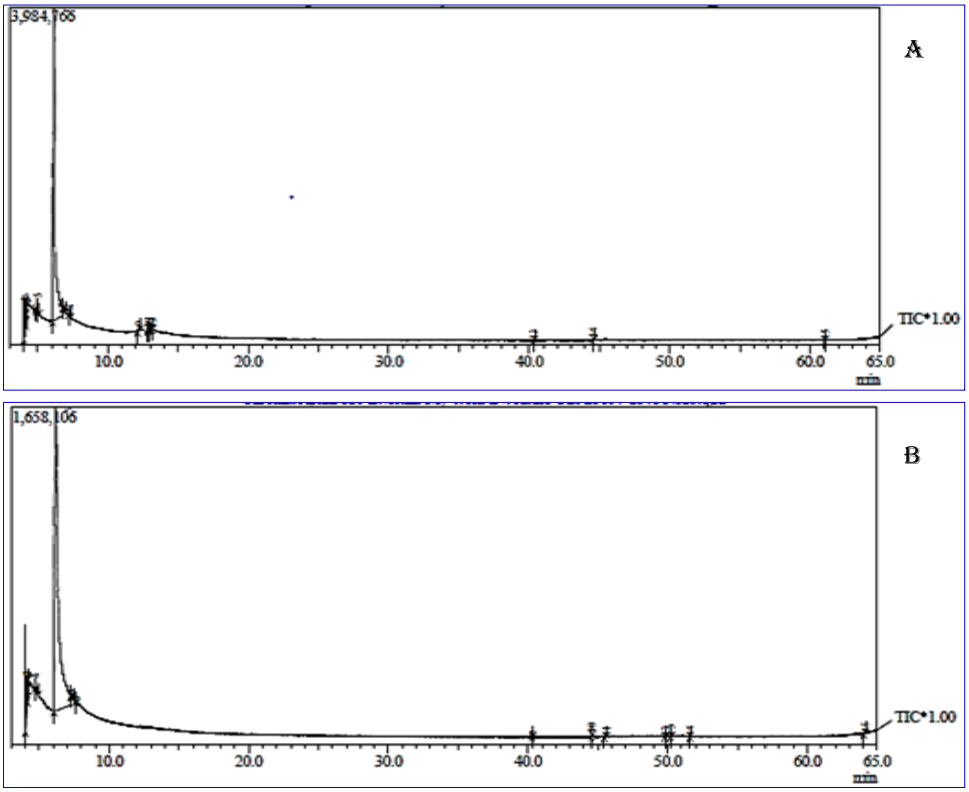

Figure (8): : GC–MS chromatogram of volatile components in methanolic extract of the studied plants. A, cultivated *Lactuca sativa;* B, wild *Lactuca serriol.*

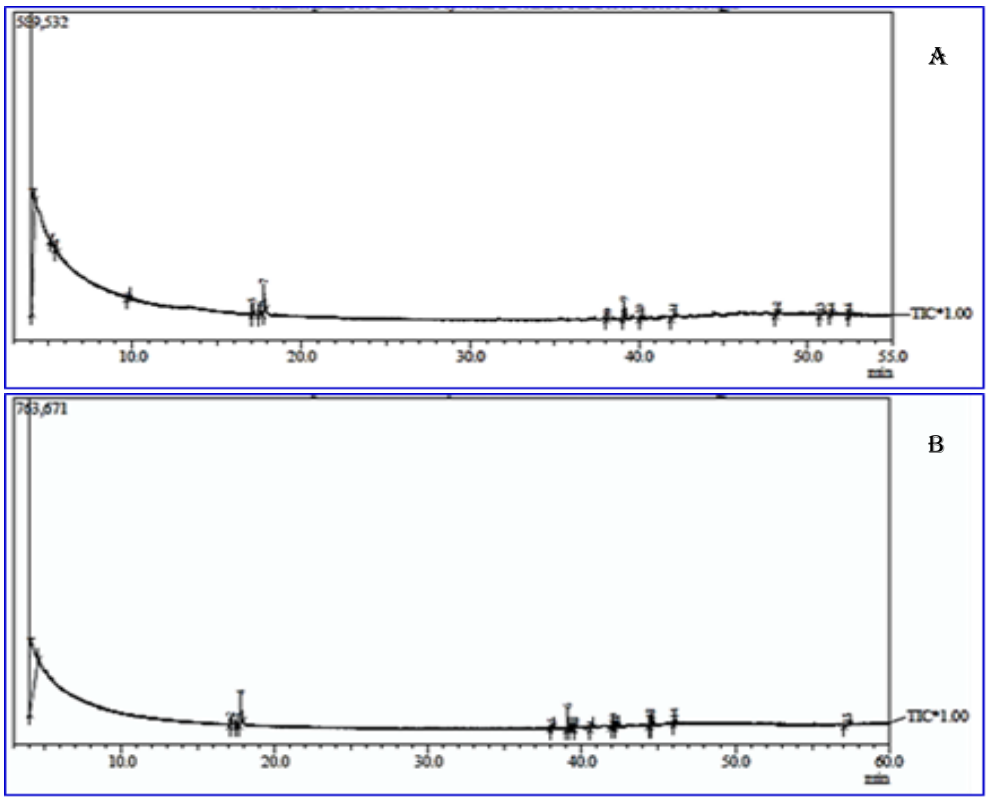

Figure (9): GC–MS chromatogram of volatile components in methanolic extract of the studied plants. A, cultivated *Brassica oleracea* methanolic extract; B, wild *Brassica tournefortii* methanolic extract

Table (8): The relative percentage of main volatile components in cultivated *Brassica oleracea* methanolic extract.

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	3-(Methylsulfinyl)propyl	4.051	783	77.018073	RI, MS
2	Acetic acid	4.406	802	0.0959802	RI, MS
3	1,3-Dioxolan-2-one	5.442	856	1.9069083	RI, MS
4	4-(Methylsulfinyl)butyl	9.682	917	0.0795265	RI, MS
5	Pentadecane	17.092	1329	1.170857	RI, MS
6	Bicyclo[2.2.1]heptan-2-one	17.496	1350	0.926082	RI, MS
7	4-Mercaptobutyl	17.773	1372	4.4209804	RI, MS
8	Pent-4-enyl	38.099	1894	0.3334423	RI, MS
9	D-Limonene	39.099	1921	7.3346144	RI, MS
10	9-Octadecene	40.056	1947	1.5706221	RI, MS
11	Dotriacontane	41.917	1973	0.0606351	RI, MS
12	2-Propenoic acid	50.738	2043	0.083183	RI, MS
13	2-Hexanone	50.717	2067	0.587968	RI, MS
14	2-Hexanol	50.932	2091	0.2400013	RI, MS
15	Indol-3-ylmethyl	52.416	2160	0.1111136	RI, MS
16	Others	-----	-----	4.059	RI, MS

Table (9): The relative percentage of main volatile components in cultivated *Brassica tourneforttii* methanolic extract**.**

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	3-(Methylsulfinyl)propyl	4.14	786	15.4728	RI, MS
2	Acetic acid, hydrazide	17.09	1438	4.51263	RI, MS
3	4-(Methylsulfinyl)butyl	17.49	1487	4.8519	RI, MS
4	4-(Methylthio)-3-butenyl	17.763	1513	19.0375	RI, MS
5	Pentadecane	38.091	1967	8.47653	RI, MS
6	Bicyclo[2.2.1]heptan-2-one	39.087	2031	37.2535	RI, MS
7	Pent-4-enyl	39.284	2045	1.04026	RI, MS
8	Triacontane	39.542	2093	1.25016	RI, MS
9	D-Limonene	40.638	2108	1.34037	RI, MS
10	9-Octadecene	42.27	2187	0.6209	RI, MS
11	Dotriacontane	42.209	2206	0.41452	RI, MS
12	2-Propenoic acid	44.419	2275	0.35951	RI, MS
13	3-Hexanol	44.609	2262	0.54389	RI, MS
14	2-Hexanol	49.913	2308	0.64554	RI, MS
15	Indol-3-ylmethyl	57.194	3463	0.89989	RI, MS
16	Others	-----	-----	32805	RI, MS

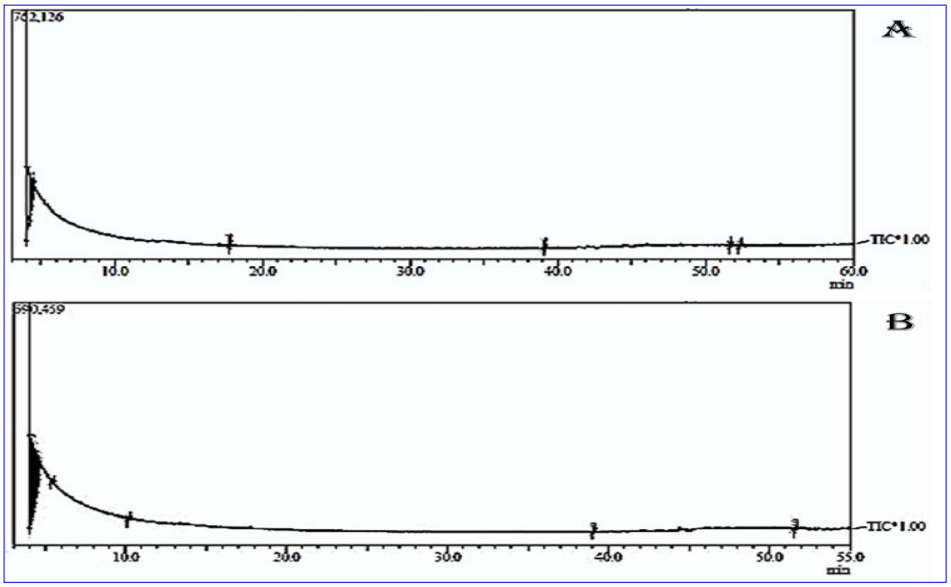

Figure (10): GC–MS chromatogram of volatile components in methanolic extract of the studied plants. A, cultivated cultivated *Raphanus sativus* methanolic extract; B, wild *Raphanus raphanistrum* methanolic extract.

Table (10): The relative percentage of main volatile components in cultivated *Raphanus sativus* methanolic extract.

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	Oleic Acid	4.087	678	32.453	RI, MS
2	Furfural	4.209	695	3.7211	RI, MS
3	Dimethyl trisulfide	4.35	712	22.0509	RI, MS
4	Propylparaben	4.61	732	21.8452	RI, MS
5	Sulforaphene	17.781	1040	2.07109	RI, MS
6	Octadecanoic Acid	39.109	1784	7.51147	RI, MS
7	Tocopherol	51.672	1945	2.83095	RI, MS
8	Dodecanoic acid	52.316	2055	1.81597	RI, MS
9	Cholesterol	52.597	2091	1.32152	RI, MS
10	Campesterol	52.638	2107	2.83849	RI, MS
11	Others	-----	-----	1.54	RI, MS

Table (11): The relative percentage of main volatile components in wild *Raphanus raphanistrum* methanolic extract.

ID	Name of the compound	Retention Time (min)	Retention Index (RI)	Peak area (%)	Identification
1	n-Hexadecanoic acid	4.082	867	54.0958	RI, MS
2	Oleic Acid	4.126	872	1.61068	RI, MS
3	Dimethyl trisulfide	4.346	894	1.07379	RI, MS
4	Furan, 2,5-dimethyl-	4.429	901	0.90391	RI, MS
5	Sulforaphene	4.521	917	7.10134	RI, MS
6	Octadecanoic Acid	4.56	954	2.64898	RI, MS
7	Tocopherol	39.104	1673	11.23626	RI, MS
8	Cholesterol	39.942	1721	19.0759	RI, MS
9	Campesterol	51.694	1956	0.91088	RI, MS
10	Others	-----	-----	1.33	RI, MS

Brassica tournefortii contains high concentration of 3-Bicyclo[2.2.1]heptan-2-one,1,7,7-trim-ethyl-37.254%), 4-(methylthio)-3-butenyl (19.04%) and (methylsulfinyl) propyl (15.4728%). For *Raphanus* species, GC-MS analysis showed the presence of 11 major peaks for *R. sativus* while in *R. raphanistrum*, 10 peaks only was detected (Figure 14). The major chemical compounds identified in *R. sativus* were oleic acid (32.45%), Dimethyl trisulfide (22.0509%) trisulfide (22.0509%) and propylparaben (21.8452%) as shown in Table (13). In comparison of the major chemical constituents in *R. raphanistrum*, n-Hexa-decaanoic acid (54.0958%), cholesterol (19.076%) and Tocopherol (11.236%) were recorded (Table 11).

The correlation coefficient among the primary constituents and secondary metabolites was detected (Figure 11). The highest positive correlation was recorded between total protein and saponins (r=0.59). However, no correlation (r=0.11) was recorded between tannins and alkaloids and between total phenols and saponins. The clustered heatmap among the studied taxa was presented in Figure (12). Priciple coordinate analysis (PCA) was shown in Figure (13) showing that the three family was interposed based on phytochemical analysis, where the highest phytochemical parameters resulted in this ordination were total carbohydrates, total flavonoids, total lipids and tannins.

DISCUSSION

Metabolism is a series of chemical events that take place in plants with the aid of enzymes, resulting in the synthesis and utilization of a wide variety of compounds, such as carbohydrates, fatty acids, amino acids, polymers, and nucleotides (polysaccharides, proteins, lipids, DNA, RNA, etc.). These activities are termed primary metabolism, and the substances that are vital to the plant's existence and play a part in sustaining the plant's viability are referred to as primary metabolites (BOCSO and Butnariu, 2022). Due to their crucial role as photosynthetic intermediates, plant tissues contain more primary metabolites than tissues from other animals (Sánchez-Mata *et al*., 2012). On the other hand, secondary metabolites are the last metabolic products without any structural significance or experimental physiological function. They are essential for the food and flavor quality of the products, as well as for technical, industrial, agricultural, pharma-ceutical, applications (Wu *et al*., 2022; Mahdi *et al*., 2023).

Aium leptophyllum has the highest concentration of total carbs and lipids, whereas *Apium gravolens* has the highest concentration of total proteins. This result is consistent with the findings of Shad *et al*. (2011), who examined the proximate composition of wild species from *Apium graveolens* and found that all the

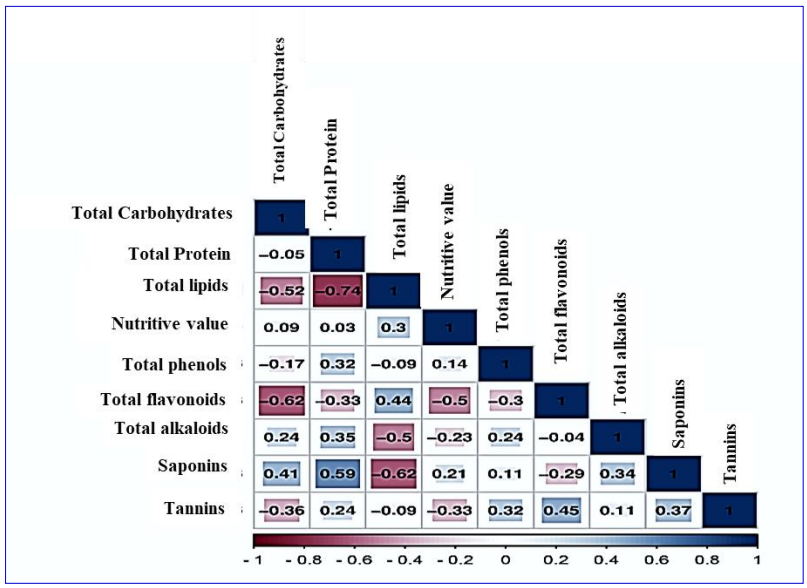

Figure (16): Correlation coefficient between different phytochemical constituents of the cultivated and their wild relative species.

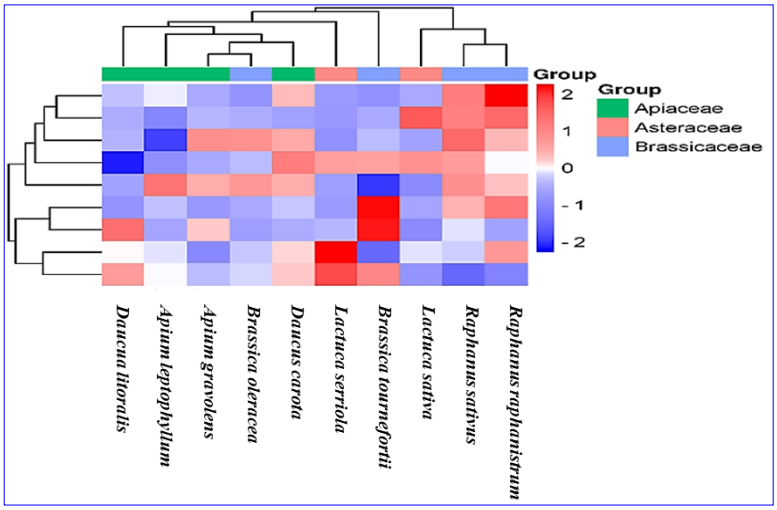

Figure (17): Color Heatmap among the studied family and phytochemical parameters.

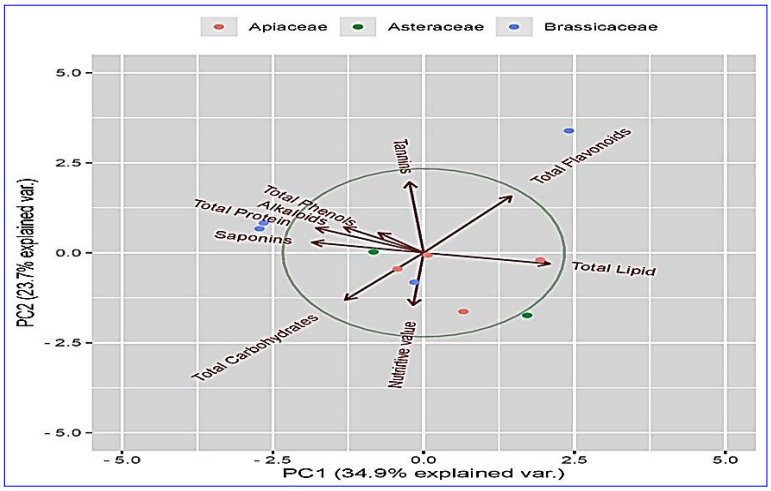

Figure (18): Principle coordinate analysis (PCA) showing the relation between the studied family and the phytochemical parameters.

examined plants contained appreciable amounts of Fat, protein, and vitamin C, with the wild celery having the highest levels of these compounds. These findings may explain why wild *Apium* species are an excellent source of proximate composition and vitamins.

The analysis of the proximate composition revealed that the aerial part of *Daucus carota* displayed the highest carbohydrate content (49.046%). This finding is consistent with a study conducted by Ayeni et al. (2018), which also reported significant levels of carbohydrates (51.81%) in *D. carota*, along with substantial quantities of protein, fibers, and lipids. These results indicate that *D. carota* could serve as an excellent food source, contributing significantly to meeting human nutritional requirements for normal growth and providing adequate protection against diseases associated with malnutrition (Asaolu et al., 2012). The presence of such diverse macronutrients underscores its potential as a valuable dietary component for promoting overall health and well-being.

Apiaceae species are regarded as the most important medicinal plant family, as they include several classes of phytochemicals with diverse biological actions. All polyphenolic chemicals, including flavonoids, tannins, alkaloids, saponins, and phenolic acids, are typically detected in extracts of apiaceae species (*Apium* and *Daucus* sp.). This conclusion is consistent with the findings of Sayed-Ahmad *et al*. (2017), who examined the phytochemical makeup of the Apiaceae family.

Lactuca serriola is regarded as the parent of cultivated lettuce (*L. sativa* L.) in the Asteraceae family since it has higher concentrations of carbohydrates, lipids, flavonoids, and tannins than *L. sativa*. This conclusion concurred with Nayeen and Imran's (2018) which found Phytochemicals of *L. serriola* contains a vast quantity of phytochemicals.

Brassica sp. has an important function in human nutrition among Brassicaceae since it contains a significant number of phytochemical substances, particularly phenolic compounds. All polyphenols are present in specific farmed and wild *Brassica*. *Brassica tournefortii* contains more phenols, tannins, and flavonoids than *B. oleraceae*. This conclusion is consistent with the findings of Tlili *et al*. (2022) who examined the biochemical composition and genetic diversity of many populations of wild *B. tournefortii*.

Raphanus raphanistrum has the largest concentrations of total protein, lipids, saponins, and tannins compared to *R. sativa*. Therefore, according to this study, wild plants are considered natural sources of phenolic compounds, particularly flavonoids, which play a crucial role in the stability of food products and are considered antioxidative defence mechanisms of biological systems (Nijveldt *et al*., 2001).

Lactuca serriola has been found to possess the highest nutritious value. Because it is a rich source of nutrients and bioactive substances that promote health, it is regarded a desirable edible plant for inclusion in a balanced and varied diet. This outcome concurs with Liberal *et al* (2021).

Significant bioactive chemicals, such as alkaloids, phenolic acids, terpenes, glycosides, and flavonoids, are primarily responsible for antioxidant activity (Al Aboody, 2020). Using the DPPH technique, the antioxidant activity of the selected plant species was determined. Ascorbic acid served as the benchmark. *Apium gravolens* has the highest antioxidant activity within the family Apiaceae (Din *et al*., 2015). *Lactuca serriola* recorded the highest antioxidant activity among Asteraceae. By assessing the radical scavenging effect on the DPPH radical, it was determined that the extract of the aerial portions of *Lactuca serriola* exhibited a high level of radical scavenging activity (Abdul-Jalil, 2020). *Raphanus sativus* exhibited the highest antioxidant activity among Brassicaceae. *Raphanus sativus* is a rich source of antioxidant chemicals and possesses high antioxidant effects, according to the results of Kim *et al*. (2016), who explored Antioxidant Activity of two cultivars of *R. sativus* and found the both cultivars have a potential source of antioxidants; therefore, *Raphanus* can be used as natural antioxidants.

Apium gravolens has the highest concentration of 1-Hexyl-2- nitrocyclohexane, whereas *A. leptophyllum* is rich in 9,7-octadecadinal, z-oleic acid, and cis-13-octadecanoic acid. It has been reported that the hexyl-2-nitrocyclohexane found only in A. *gravolens* has anti-inflammatory, neuroactive, and analgesic properties (Dinesh *et al*., 2014). According to Selvamangai and Bhaskar (2012), 1-Hexyl-2-nitrocyclohexane is a ketone with antibacterial, antioxidant, and anti-inflammatory properties. In general, octadecanoic acid or n-alkanes possess significant antibacterial properties (He, 2009). *Daucus carota* is rich in Oxalic acid, cyclobutyl isohexyl, and Acetyl valeryl ester, but *D. litoralis* is rich in Ethanol, 2,2-oxybis-, diacetate. According to numerous studies, the Apiaceae family is a rich source of aromatic and chemical antioxidants, particularly phenolic compounds. These natural chemicals have numerous biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis, and anticancer properties (Thiviy *et al*., 2021). Hexadecanoic acid and 3-Methyl heptane are the major chemical components in both *lactuca* sp. and Asteraceae. n-hexadecanoic acid may function as an anti-inflammatory agent and have antibacterial and antifungal properties (Aparna et al., 2012).

Brassica oleracea had the highest concentration of 3-(Methylsulfinyl) propyl, whereas *B. tournefortii* had the highest concentration of Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-. This result varies from that of Kumar *et al*., (2017), who examined the Volatile Compounds of two *Brassica rapa* varieties using GC-MS and discovered that Dodecane (1.47%), Heneicosane (1.23%), and Hexadecane (1.15%) were the most abundant components. *Raphanus sativa* is rich in oleic acid, whereas *R. raphanistrum* is rich in n-hexadecanoic acid. Oleic acid has a wide range of physiological functions and a positive impact on

cancer, autoimmune, and inflammatory illnesses (Song *et al*., 2019).

CONCLUSION

The present study highlights the presence of significant medicinal compounds in both cultivated taxa and their wild counterparts, as evidenced by phytochemical screening and gas chromatography mass spectrometry analysis. Phenols, flavonoids, alkaloids, tannins, and saponins were detected in both plant groups, indicating their potential therapeutic value. Notably, wild plants exhibited higher nutritive value compared to cultivated species. Additionally, wild species demonstrated prominent antioxidant activity. These findings suggest that harnessing the potential of wild plants as alternative crops could be advantageous amidst ongoing climate change challenges. Wild plants serve as crucial natural reservoirs of phenolic compounds, which are well-known for their antioxidative defense mechanisms in biological systems and potential applications in food preservation. Moreover, this study provides valuable insights into plant breeding programs and efforts aimed at improving crop quality by offering comprehensive data on desirable traits that can drive future advancements in agriculture.

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دراسه مقارنة بين بعض الأنواع المزروعة وأقاربها البرية في مصر باستخدام التركيب الكيميائي النباتي والقيمة الغذائية ونشاط مضادات الأكسدة وتحليل كروماتوغرافيا الغاز - مطياف الكتلة

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الملخص العربـــــي

يعتبر تغير المناخ من اهم العوامل التي تشكل تهديدًا رئيسيًا علي الإنتاجية الزراعية في جميع أنحاء العالم. لهذا ركزت استراتيجيات التربية الحديثة على تحسين صفات المحاصيل من خلال ادراج الأقارب البرية للمحاصيل ((CWRs -وهي أسلاف برية ترتبط ارتباطًا وثيقًا بالمحاصيل - هو خيار مستدام لتنمية المحاصيل في سياق المخاوف المناخية المستمرة. من أجل توفير بيانات فعالة وزياده الكفاءة الزراعية للمحاصيل ، تهدف هذه الدراسة إلى توفير بعض الخصائص الكيميائية النباتية والسمات البيوكيميائية لبعض النباتات المزروعة وأقاربها البرية التي تنتمي إلى ثلاث عائلات ؛ Apiaceae و Asteraceae و Brassicaceae. وفقًا لنتائج الفحص الكيميائي النباتي. يحتوي (Asteraceae) Wild *Lactuca serriola* على أعلى كثافة مغذية (457.21 كيلو كالوري / 100 جم) بينما يحتوي *Apium leptophyllum* على أقل كثافة (381.94 كيلو كالوري / 100 جم). بمقارنة قيم IC50 ، فإن *Daucus carot*a يحتوي على أدنى مستوى (0.052 مجم / مل) ولوحظ ان *Lactuca serriola* أكبر نشاط مضاد للأكسدة (0.457 مجم / مل). أوضحت هذه الدراسة أن النباتات البرية بشكل عام لها أعلى قيمة غذائية مقارنة بالأنواع المزروعة. كل هذه البيانات أوصت باستخدام الأنواع البرية كمحاصيل بديلة بدلاً من الأنواع المزروعة الشائعة خاصة في ظل ظروف التغير المناخي في الوقت الحاضر. لذلك ، تعتبر النباتات البرية مصادر طبيعية لمركبات الفينول ، والتي تعتبر آليات دفاع مضادة للأكسدة للأنظمة البيولوجية وقد تلعب دورًا مهمًا في تحسين صفات المحاصيل وتربية النباتات.