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Phytochemical Investigation and Antioxidant Activity of *Onopordum acanthium L.* in Different Regions of Western Iraq Using GC-MS Analysis

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ABSTRACT: The present study undertakes the phytochemical analysis of *Onopordum acanthium L.*, commonly named Scotch thistle, collected in year 2024 for five areas in the western region of Iraq, for its antioxidant activities. Consequently, an attempt to identify the chemical composition of the plant for Al-Karmah, Ramadi, Hit, Fallujah, and Al-Qaim, respectively, was stated on the strength of Gas Chromatography-Mass Spectrometry technique (GC-MS) technology. The outcomes showed a region-inducing, high variability in the chemical profile of this plant with active compounds in the range of 18 to 29, which is the highest number in Hit and Al-Karmah, indicating the richer biodiversity or favorable environmental conditions of the area. Compounds detected in all the regions included neovitadiene and hexadecanoic acid methyl ester, which are critical building blocks of the plant's metabolic framework for survival at different scales. Antioxidant activity of *O. acanthium* was also performed, and the plant sample from Ramadi presented the highest scavenging activity, 86% ± 0.05, reflecting higher antioxidant potential. In contrast, the lowest activity was found for the samples from Fallujah, 51% ± 0.32, probably due to less appropriate environmental conditions. The present study underlines the importance of regional environmental factors affecting the biosynthesis of secondary metabolites in *O. acanthium*. Consequently, such environmental conditions have a pronounced impact on the antioxidant properties of this plant, which might be of interest for pharmacy and agriculture. Also, the presence of ascorbic acid in the samples shows that this plant can be very useful for natural antioxidants; thus, other studies could be developed from its medicinal and nutritional use.

Keywords: *Onopordum acanthium*, Phytochemical analysis, Antioxidant activity GC-MS, Regional variation, Environmental impact

1. INTRODUCTION

Plants have traditionally played a very important role in medicine for many cultures and have served as a rich source of therapeutic agents (1). The genus Onopordum (Asteraceae) consists of approximately 50 species distributed across continental Europe, Central Asia, and Southwest Asia. Among them, *Onopordum acanthium L.*, Scotch thistle, is a tall 3-meter-high biennial; the plant has white lanate stems with spiny margined wings, and flowers are purple-colored with a panicle shape (2). Being highly aggressive, *O. acanthium* is considered a competitive species against forage plants, creating barriers to the movement of livestock. It has also been classified as an agricultural pest in countries like the US, Canada, and others.

According to KILIÇ & YILDIRIMLI, 2022(3), in China, *O. acanthium* can be found in a wide range of regions and is dominant in the Tianshan Mountains and the Junggar Basin, Xinjiang Province, around Northwest China. This region

is also considered an arid or semi-arid area (4). Traditionally, *O. acanthium* has been widely used in treating cancers, inflammatory diseases, cardiovascular diseases, and urogenital diseases all over the world (5). Particularly, among all, Cardiodoron is the famous medicinal product extracted from *O. acanthium* and has been proven effective and well tolerated in functional cardiovascular conditions, yielding favorable results in clinical practice (6).

Phytochemical studies have revealed allelochemicals from *O. acanthium* with phytotoxic activity, including flavonoids and sesquiterpene lactones, explaining the plant's widespread presence across several continents (7). Flowers from *O. acanthium* are also used in the cheese-making industry as a coagulant, and young shoots and first-year roots are eaten raw in salads (8). Besides, oil extracted from O. acanthium could be used as a biofuel and chemical feedstock; some literature has reported its fatty acid composition, tocopherol content, and mineral composition (9). Essential oils of O. acanthium plants have traditionally been used in medicine as a bactericide, cardiotonic, hemostatic, and diuretic in the treatment of nervous disorders, tumors, and ailments of the bladder, respiratory, and urinary systems. Saponins, alkaloids, sesquiterpene lactones, flavonoids, triterpenes, sterols, phenolic acids, coumarins, inulin, soluble sugars, proteins, and oils have been isolated from the species (10).

The pharmacological studies indicate that *O. acanthium* has a wide range of bioactivities, including antibacterial, antioxidant, anticancer, anti-inflammatory, analgesic, antipyretic, hypotensive, antiepileptic, wound healing, xanthine oxidase inhibitory activities, and ACE inhibitory activities (11). This immense study on the phytochemical and pharmacological properties indicates that *O. acanthium* is a plant with immense potential for traditional and current medicines (12). The present study, therefore, focuses on determining the phytochemical and antioxidant activities of *O. acanthium L.* from five regions in Western Iraq, considering how regional variations might influence the plant's bioactive compounds due to environmental factors. Its possible applications in pharmaceutical and agricultural fields are closely related to these factors.

2.MATERIALS AND MTHODS

2.1 Environmental study

A survey was conducted in Anbar Governorate, located in western Iraq, during the current year **2024** for five areas: Al-Karma, located within 43.9317230° longitude and 33.4584587° latitude; Fallujah, located within 43.8134739° longitude and 33.3672801° longitude, and Ramadi, 33.3451002° longitude. 4920297, Hit, located within 42.8823893° longitude and 33.5803759° latitude, and Al-Qaim, located within 41.1198602° longitude and 34.3506543° latitude, to study the effect of environmental diversity on the physicochemical characteristics of cotton thistle soil. Five soil samples were taken randomly from a depth of 40 cm because that is the adequate depth for the roots of most plants from each of the study sites.

2.2 Preparation of Ethanolic Extract

The extract was prepared after drying the plant in the sun, then drying it in an oven at a temperature of 60 ^{0}C, then grinding the plant using a laboratory mill after taking a weight from the ground plant (100 grams) and adding ethanol alcohol to it at a concentration of 70% in a ratio of 1:5 and using a mixer to homogenize it for 24 hours, then the filtration process using special filtration cloth, then after the solvent was removed using a rotary evaporator, the remainder was dried in a drying oven at a temperature of 60 ^{0}C , and the final dry extract was obtained, which was used in other tests (8).

2.3 Diagnosis of *O. acanthium* leaves using GC-MS technology

The compounds of the plant extract are identified by dissolving 20 g of plant powder of leaves in 200 ml of 80% ethanol, and then the chemical compounds of the leaf samples are examined using a chromatography device connected to a mass spectrometer (Gas Chromatography-Mass Spectrometry technique) and equipped by the American company of Japanese origin according to the specifications (Agilent Co- GC 7890A) (13).

2.4 Antioxidant Activity

The radical scavenging activity against the stable DPPH radical was measured using the methods described by Sharma and Bhat (2009)(14), with some modifications for microplate applications. The antioxidant activities of the plant extracts were determined based on the radical scavenging effect of the stable DPPH free radical. The assay mixture consisted of 6 µL of plant extract solution at varying concentrations, 50 µL of DPPH ethanol solution (200 µM), and 144 µL of 99% ethanol. After thorough vortexing, the mixture was allowed to stand for 25 minutes at room temperature, the absorbance was then measured at 517 nm using a microplate reader spectrophotometer, a concentration range of 0.01–3.75 µg/mL for ascorbic acid was employed as a positive control. The DPPH radical scavenging activity of each sample was expressed as the IC50 value, which was calculated from the dose–response inhibition curve (15).

2.5 Statistical analysis

Statistical analyzes were performed using SPSS v.22 and Excel 2013. Quantitative data were described using means and standard errors (mean ± standard error). The correlation coefficient (R) is calculated to determine the nature and strength of the relationship between variables. The significance level of the test is $P < 0.05$.

2. RESULTS AND DISCUSSION

3.1 Analysis *O. acanthium* leaves using GC-MS technology

Tables (1) showed a GC-MASS analysis of the vegetative part of the *Onopordum acanthium* plant for five regions in Anbar, namely (Al-Karmah, Ramadi, Hit, Fallujah, and Al-Qaim). By starting with the number of active compounds, the current study can quickly identify the regions that have the greatest amount of chemical diversity. This helps to focus on areas with the most important ecological, pharmaceutical or agricultural value, and areas with a greater number of active compounds may indicate richer biodiversity or more important environmental factors influencing the chemical composition of plants. Prioritizing these regions can lead to more impactful research outcomes. Once regions are prioritized based on the number of compounds, further sorting by type of active compounds provides deeper insights into the specific chemical profiles of each region.

From the tables it is possible to see areas of high impact and areas with a higher number of compounds are likely to have a wider range of potential applications, from pharmaceuticals to agriculture. Focusing on these areas first can maximize the impact of research efforts. Many compounds can also indicate a healthy and diverse ecosystem, which is critical to conservation efforts. This detailed analysis can reveal the functional roles of compounds specific, their potential benefits, and how they interact within the ecosystem.

Table 1: Compounds effective in the plant in the Qarma area

			the Qarma area		
Number	RT (min)	Area	Name	Quality	CAS Number
1	6.676	1.40	1-Butanol, 3-methoxy-	42	002517-43-3
2	15.118	0.60	Chloroacetic acid, 2-ethylhexyl ester	80	005345-58-4
3	18.423	0.43	2,4-Di-tert-butylphenol	46	000096-76-4
4	24.094	3.24	Tetradecanal	94	000124-25-4
5	24.753	0.79	Neophytadiene	99	000504-96-1
6	25.153	0.24	Citronellyl isobutyrate	72	000097-89-2
7	25.454	0.48	Neophytadiene	46	102608-53-7
8	25.962	0.52	Hexadecanoic acid, methyl ester	95	000112-39-0
9	26.439	2.37	1,1'-Butadiynylenedicyclohexanol	35	038463-39-7
10	26.585	4.44	Tridecanoic acid	90	000638-53-9
11	27.072	0.40	Hexadecanoic acid, ethyl ester	96	000628-97-7
12	27.15	1.68	Trioxsalen	41	003902-71-4
13	29.205	2.28	Linolenic acid	96	000463-40-1
14	29.537	0.87	Cycloundecene, 1-methyl-	50	088828-82-4
15	29.589	0.96	.Ethyl 9α-linolenate	95	001191-41-9
16	29.76	3.41	Formamide, N-(1-cyanoethenyl)	47	303124-14-3
17	30.404	2.06	Eremanthin	53	019213-99-1
18	30.585	0.86	Doconexent	43	138146-05-1
19	30.86	7.02	Columbin	30	019456-19-0
20	31.234	3.89	Eremanthin	49	003102-70-3
21	33.237	7.62	Norethindrone	35	008986-07-0
22	33.506	7.44	Retinol, acetate	38	1000299-37-8
23	36.645	12.30	Methyl 4,7,10,13,16-docosapentaenoate	44	039599-18-3
24	36.967	21.20	Ethyl 5,8,11,14-eicosatetraenoate	35	1000223-36-2
25	43.209	1.15	Eicosane	78	000112-95-8
26	44.117	1.69	.gamma.-Sitosterol	41	000083-47-6
27	44.569	1.73	.beta.-Amyrin	96	000559-70-6
28	45.274	8.07	Lupeol	49	000545-47-1
		99.12			

Table 2: Compounds effective in plants in the Fallujah area

			the Fallujah area		
Number	RT (min)	Area%	Name	Quality	CAS Number
1	6.692	3.77	1-Butanol, 3-methoxy-	38	001072-40-8
2	15.134	0.94	Chloroacetic acid, 2-ethylhexyl ester	91	005345-58-4
3	18.428	1.61	2,4-Di-tert-butylphenol	70	000096-76-4
4	24.115	5.01	Hexadecanal	93	000629-80-1
5	24.753	1.68	Neophytadiene	87	000504-96-1
6	25.153	0.70	Neophytadiene	58	1000314-60-8
7	25.454	0.88	Phytol, acetate	49	076337-16-1
8	25.973	3.81	Hexadecanoic acid, methyl ester	99	000112-39-0
9	28.52	1.70	9,12-Octadecadienoic acid, methyl ester	91	002462-85-3
10	28.577	3.92	.Linolenic acid, methyl ester	98	000301-00-8
11	29.107	3.64	Heptadecanoic acid, 9-methyl-, methyl ester	95	005129-61-3

12	29.537	0.64	3-Heptyne	46	002586-89-2
13	29.594	1.46	Doconexent	91	1000336-77-4
14	29.786	9.01	Ambrosin	47	1000196-17-2
15	33.268	3.63	Methyl 4,7,10,13,16,19-docosahexaenoate	30	1000327-64-0
16	33.532	8.30	(E)-4,8-Dimethylnona-1,3,7-triene	38	019945-61-0
17	37.014	5.00	: Methyl 4,7,10,13,16-docosapentaenoate	38	1000244-64-9
18	43.194	2.67	Stigmasterol	43	1000258-63-4
19	44.102	5.00	β-Sitosterol	45	1000244-64-9
20	44.564	5.50	.beta.-Amyrin	95	000559-70-6
21	45.269	30.90	Lupeol	38	1000351-62-2
		99.77			

Table 3: Compounds effective in plants in the Ramadi area

the Ramadi area

Number	RT (min)	Area %	Name	Quality	CAS Number
1	15.139	4.54	1-Hexanol, 2-ethyl-	78	005345-58-4
2	18.428	0.79	aR-Himachalene	58	019419-67-1
3	24.11	6.14	Tridecanal	91	010486-19-8
4	24.753	2.35	Neophytadiene	91	000504-96-1
5	25.142	0.59	1,4-Eicosadiene	58	1000131-16-3
6	25.454	1.00	Neophytadiene	52	102608-53-7
7	25.526	0.70	Caryophyllene oxide	38	024230-79-3
8	25.962	5.36	Hexadecanoic acid, methyl ester	99	000112-39-0
9	28.51	1.13	9,12-Octadecadienoic acid, methyl ester	96	002462-85-3
10	28.577	8.44	Linolenic acid, methyl ester	99	000301-00-8
11	29.101	3.03	Methyl stearate	96	000112-61-8
12	29.786	6.95	Ambrosin	35	1000099-25-4
13	33.247	6.65	benz[d]isozazole, 3-ethyl-	27	066033-77-0
14	33.522	11.54	1-Bromo-11-iodoundecane	30	139123-69-6
15	36.998	10.46	Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	25	002158-89-6
16	43.199	3.25	d-Mannitol, 1-decylsulfonyl-	64	1000316-02-1
17	44.117	4.29	Methyltris(trimethylsiloxy)silane	50	017928-28-8
18	44.553	4.50	Silicic acid, diethyl bis(trimethylsilyl) ester	47	003555-45-1
19	45.254	11.26	Tetrasiloxane, decamethyl-	53	000141-62-8
20	47.049	7.02	Benzo[h]quinoline, 2,4-dimethyl-	58	000605-67-4

Table 4: Compounds effective in plants in the Hit region

M4 Hit region

Number	RT (min)	Area%	Name	Quality	CAS Number
1	15.118	0.64	2-Undecenal	58	003883-58-7
2	18.091	0.60	Cadina-1(10),6,8-triene	81	001460-96-4
3	24.094	3.39	Tetradecanal	96	000124-25-4
4	24.748	0.97	NEOPHYTADIENE	99	000000-00-0
5	25.153	0.31	1-Methoxy-3-(2-hydroxyethyl) nonane	41	000000-00-0
6	25.454	0.42	Phytol, acetate	87	102608-53-7

7	25.532	0.66	Non-1-en-8-yn-5-one	38	000000-00-0
8	25.962	1.05	Methyl palmitate	97	000112-39-0
9	26.44	1.97	Ectylurea	30	000095-04-5
10	26.585	2.44	4-(N-Isopropylamino)-6-phenylpyridi-2-one	83	000000-00-0
11	27.073	0.28	Ethyl palmitate	94	000628-97-7
12	27.15	1.15	Dibenzothiophene-1-carboxylic acid	90	034724-68-0
13	28.515	0.39	Methyl linoleate	95	000112-63-0
14	28.572	1.42	Methyl linolenate	99	000301-00-8
15	28.925	0.51	trans-Phytol	83	000150-86-7
16	29.527	0.53	Linoleic acid ethyl ester	90	000544-35-4
17	29.589	0.49	(E,Z)-1,5-Cyclodecadiene	89	001124-78-3
18	29.755	3.60	Ambrosin	55	021064-19-7
19	30.398	1.33	Benzene, 2-methoxy-1,3,4-trimethyl-	53	021573-36-4
20	30.834	10.89	Methyl 5-hydroxymethyl-2-furoate	41	036802-01-4
21	31.223	2.78	Eremanthin	60	041807-15-2
22	33.226	5.19	cis-Testosterone	46	000000-00-0
23	33.496	11.68	BROMCYCLOPENTAN	38	000137-43-9
24	36.64	6.88	Ethyl phenethyl alcohol	50	041673-72-7
25	36.972	25.35	Benzeneethanol, ar-ethyl-	42	041673-72-7
26	43.204	1.33	Hydromethylsiloxane	53	001873-88-7
27	44.102	2.03	Stigmasterol, 22,23-dihydro-	95	000000-00-0
28	44.558	2.10	Aristolone	70	006831-17-0
29	45.259	9.63	Lupeol	55	000545-47-1

Table 5: Compounds effective in plants in the Al-Qaim region

Number	RT (min)	Area %	Name	Quality	CAS Number
1	7.641	0.46	2-Ethylhexanol	80	000104-76-7
2	15.144	2.48	1-PENTENE, 2-ETHYL-4-METHYL-	46	003404-80-6
3	18.418	2.39	Phenol, 3,5-bis(1,1-dimethylethyl)-	81	001138-52-9
4	24.12	5.50	Oxirane, hexadecyl-	87	007390-81-0
5	24.748	1.42	citronellyl 3-methylbutanoate	38	000000-00-0
6	25.09	0.33	Cyclononasiloxane, octadecamethyl-	37	038147-00-1
7	25.147	0.48	Cyclodecene, 1-methyl-	38	066633-38-3
8	25.448	0.54	.beta.-Citronellol	49	000106-22-9
9	25.972	7.01	Methyl palmitate	97	000112-39-0
10	28.52	1.32	Methyl linolelaidate	94	002566-97-4
11	28.582	5.27	Linolenic acid, methyl ester	98	000301-00-8
12	29.106	5.94	Methyl stearate	98	000112-61-8
13	29.807	5.99	Ambrosin	49	000000-00-0
14	33.252	8.97	1-O-Tolylprop-2-en-1-one	38	039627-60-6
15	33.532	13.05	Prenyl bromide	43	000870-63-3
16	35.758	14.30	Nonane, 3-methyl-	35	005911-04-6
17	36.983	16.08	Testolactone	58	070311-96-5
18	45.264	4.38	Cyclotrisiloxane, hexamethyl-	58	000541-05-9

Table 6 shows the regions according to the number of active compounds, and they included Karma: 28, Fallujah: 21, Ramadi: 20, Hit 29, and Al-Qaim: 18. In the order of these regions, the current study find that Hit and Qarma have the largest number of active compounds, followed by Fallujah, Ramadi, and Al-Qaim, according to the results. Based on the number of active compounds, this ensures that research efforts and resource allocation are focused on areas with the

greatest potential for important results and applications. Spectroscopic analysis by type of active compounds then allows detailed and targeted analysis, providing comprehensive insights into the chemical environment of each region. This approach increases the efficiency and impact of research efforts.

Table 6: Number of compounds resulting from GC-MASS analysis in each study area

Area	Number of compounds
Alkarma	28
Alfaluwja	21
Alramadi	20
Hit	29
Al-Qaim	18

Table 7 below highlights the common compounds found in *O. acanthium* in five different regions of Iraq: Al-Qurma, Fallujah, Ramadi, Hit, and Al-Qaim. This comparative analysis reveals the distribution and prevalence of some bioactive compounds within plant species in these diverse geographic regions.

1-Butanol-3-methoxy: This chemical is available in the Karma and Fallujah areas while it is absent in the regions of Ramadi, Hit, and Al-Qaim. Its limited distribution contrariwise points to favorable environmental conditions for synthesis or accumulation in Qurma and Fallujah.

2-Chloroacetic acid 2-ethylhexyl ester: It is present in Karma, Fallujah, and Ramadi, and it follows that the presence of this chemical in three regions shows a wider environmental range. Its absence in Hit and Al-Qaim may indicate differences in land chemistry or micro-climatic conditions.

3-24-Di-tert-butylphenol: This metabolite is only present in the Karma and Fallujah samples; therefore, its expression is influenced either by the nutrients within the soil or by the prevailing pH in both locations.

4-Neovitadiene: It has a wide distribution, appearing in Karma, Fallujah, Ramadi, and Hit. This wide distribution suggests that these are core plant metabolites, which may play a role in the adaptation and survival of the plant in different environments.

5-Hexadecanoic acid methyl ester: Detected throughout all five zones, its ubiquitous presence highlights its importance in the plant's metabolic processes. The substance's abundance in these varied ecological areas indicates its vital function in the plant's development and survival strategies.

6-Linolenic acid methyl ester: Existent in Fallujah, Ramadi, Hit, and Al-Qaim, but entirely absent in Al-Karma. Lack of its production in the latter could be due to some local deficiencies or stresses in the environment inhibiting its production altogether.

7-Methyl stearate: Present in the two provinces of Ramadi and Al-Qaim. Its identical appearance in the mentioned provinces shows special climatic or soil conditions that enable such production.

8-Ambrosin: In Fallujah, Ramadi, Hit, and Al-Qaim, this may point to its importance in mechanisms of plant adaptation to different environmental conditions.

9-Stigmasterol: Found in Fallujah and in Hit; these can be related to some physiological or defense properties within the plant in that region under specific conditions. 10-Beta.-Amirin: It occurs in Karma and Fallujah. The small dissemination of it testifies to special ecological or evolutionary pressures in the respective areas, which give this compound an advantage in its biosynthesis pathway. 11-Lubeol: It has been reported from Karma, Fallujah, and Hit. The presence of

lubeol suggests that it may have an important role in the metabolic pathways of these plants, probably conferring protective benefits against some forms of environmental stresses.

The distribution of such compounds would further educate ecological and environmental factors that influence the biosynthesis of secondary metabolites in Onopordum acanthium. The fact that some important compounds, like neovitadiene and hexadecanoic acid methyl ester, are represented in more than one region might point to their being part of the plant's metabolic framework necessary for its adaptation to living conditions. On the other hand, the inability to detect some of these compounds in one region or another shows that local environmental conditions truly affect the active chemical profile of this plant. One example of a specific environmental stress or soil condition that may impede production is the non-detection of linolenic acid methyl ester in the vine. In this regard, it will perform a comparative analysis, emphasizing the possibility of understanding how environmental factors interact with plants to affect their chemistry. These results can be helpful in further studies related to the adaptation possibility of the plant Onopordum acanthium regarding the variation in the environment and its potential pharmaceutical and agricultural uses(16, 17).

Table 7: Replication of some effective compounds between study areas

Compound Name	Qarma	Fallujah	Ramadi	Hit	Al-Qaim
1-Butanol 3-methoxy-	Yes	Yes	No	No	No
Chloroacetic acid 2-ethylhexyl ester	Yes	Yes	Yes	No	No
24-Di-tert-butylphenol	Yes	Yes	No	No	No
Neophytadiene	Yes	Yes	Yes	Yes	No
Hexadecanoic acid methyl ester	Yes	Yes	Yes	Yes	Yes
Linolenic acid methyl ester	No	Yes	Yes	Yes	Yes
Methyl stearate	No	No	Yes	No	Yes
Ambrosin	No	Yes	Yes	Yes	Yes
Stigmasterol	No	Yes	No	Yes	No
beta.-Amyrin	Yes	Yes	No	No	No
Lupeol	Yes	Yes	No	Yes	No

Identification has a lot of advantages over the conventional morphological approaches. The key applications of spectroscopy to plant classification comprise the following: Spectroscopic techniques such as FTIR and NIR, providing complex chemical fingerprints of plant tissues. Such spectral fingerprints represent the varying mixture of biochemical compounds deposited within plant tissues of different species and can, therefore, be applied in distinguishing similar taxa (18).

The spectroscopic methods also provide for fast non-destructive analysis of the plant samples. They are, thus, suitable for screening and classification on large scales because of this. Cases where one needs to analyze samples of rare herbs and plants, whereby destructive sampling is just not possible, make a whole lot of difference. As such, Li et al. (2021)(16) explained that the chemical profile obtained by spectroscopy enables the chemical classification to supplement the traditional morphological approaches when these morphological differences are so minute or ambiguous, as stated by (Younis et al. 2022)(19).

Spectroscopic data paired with chemical analysis helps pinpoint where plant samples come from. This has an impact on documenting medicinal herbs checking the source of farm products, and exploring plant biogeography (20). Chemospectral imaging and other spectroscopic methods are now more common in high-speed plant phenotyping. These tools let scientists assess different plant traits and group plants. Given these features, spectroscopy can spot quick shifts

in plant biochemistry caused by environmental stress. This might allow scientists to classify plants based on how they react to stress or how they've adapted to various settings (20).

The data from plant chemistry spectra has an influence on research into evolution and species relationships. When combined with other data types, like genetic and morphological info, it helps to build a more complete and precise taxonomy. Spectroscopic analysis, or spectrometry, has caused a revolution in plant classification moving it towards more objective and quantitative methods (21). These methods give deep insights into plant biochemistry and add new angles to our grasp of plant relationships and diversity working alongside traditional morphological approaches. As tech gets better, spectroscopy will become more crucial to plant staging and taxonomy (22).

3.2 Antioxidant Activity of *Onopordum acanthium*

The current study on the oxidative activity in Onopordum plants originating from different regions of Anbar Governorate, western Iraq, as shown in Table 8, depicts great variation within the scavenging activity, ascorbic acid concentration, and standard deviation values, hence giving insight into the plant's antioxidant potential within those areas. Free radical scavenging activity expressed in percentages varies significantly among all the regions. Results clearly revealed that the scavenging activities by the plant samples of Ramadi recorded the highest, with a value of 86% ± 0.05, hence indicating the possibility for better antioxidant activity in this region.

This has a lot to do with what's going on in the environment, what's in the soil, or specific weather patterns that help the plant build up more antioxidants. These things all play a part in making the plant store up these helpful compounds. After Ramadi, samples taken from Hit showed significant litter activity of 72% ± 0.93. This value, although lower than that of grey, still indicates a strong presence of antioxidants. Al-Qaim and Karma areas witness moderate littering activities amounting to 66% ± 0.11 and 57% ± 0.62, respectively. These values indicate that although these areas also support the growth of antioxidant-rich *O. acanthium*, the concentration of active compounds may be affected by slightly different environmental factors such as temperature, soil type, neighboring plants and the nature of the soil microbes. Interestingly, samples taken from Fallujah showed the lowest activity at 51% ± 0.32. This large difference in antioxidant potential could be a result of various factors such as pollution levels, soil degradation, or less ideal growing conditions, which may affect the biosynthesis of antioxidant compounds in the plant. In addition, the ascorbic acid concentration was measured at 81 mg/mL ± 0.22, to serve as a reference point for the total antioxidant capacity of plant samples. The high concentration of ascorbic acid combined with its scavenging activity highlights the powerful antioxidant properties of Onopordum, which can be utilized in medicinal or nutritional applications, and supports statistical analysis to determine the strength and nature of the relationship between variables. The results were considered statistically significant at a p-value of < 0.05, indicating that the observed regional differences in scavenging activity are unlikely to be due to chance.

As a general condition, the oxidative activity of O. acanthium plants originating from various regions highly varies; therefore, Ramadi has the highest antioxidant potential. These differences clearly indicate environmental effects on the biosynthesis of antioxidant substances in this plant. However, high ascorbic acid contents point out that this plant may be a useful natural antioxidant source that needs further investigation in relation to its health and nutrition value, as illustrated in Table 8.

Table 8: The antioxidant activity of *Onopordum acanthium*

No.	Scavenging (%)
alkarma	$57^{af}\pm 0.62$
alfaluwja	$51^{b}\pm 0.32$
alramadi	$86^{c}\pm 0.05$
hit	$72^{d}\pm 0.93$
Al-Qaim	$66^{ab}\pm 0.11$
Ascorbic (mg\ml)	$81^{f}\pm 0.22$
$LSD_{0.05}$	3.547

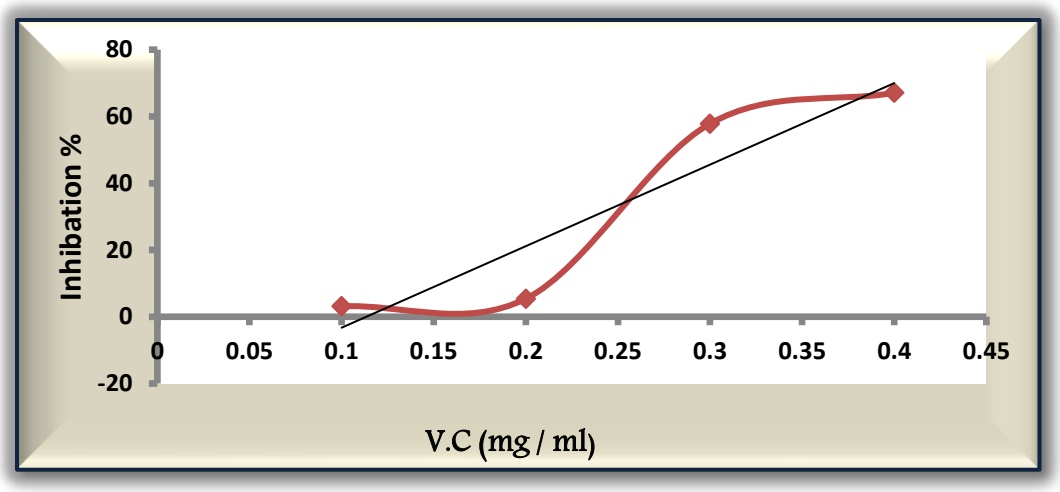

Figure 1: Show the curve of Ascorbic (V.C)

The plant has a wealth of bioactive compounds like flavonoids phenolic acids, and tocopherols that show antioxidant activity(23, 24). Due to its antioxidant properties, these compounds might help to neutralize free radicals and cut down on oxidative stress. Research aimed to examine the in vitro antioxidant activity of *O. acanthium* extract discovered that the extract had potential to scavenge free radicals through the DPPH test (25). This points to the plant's ability to combat oxidative damage. Multiple studies have indicated that *O. acanthium* extract might reduce oxidative stress and tissue damage through its phytochemical activities (26). Scientists noticed effects that protect against oxidative stress in diabetic rats within pancreatic beta cells and cardiac tissues, and they link the antioxidant activities of *O. acanthium* to its uses in traditional medicine for ailments like inflammation and high blood pressure. The plant's antioxidant properties play a big part in its healing effects. Since this property is of prime importance in the applications of Acanthium as a medicinal agent, standardization studies have been done to evaluate and quantify its antioxidant activity (27).

Oueslati et al. (2019)(28) showed that *O. acanthium* seed oil is a by-product rich in polyphenols with remarkable antioxidant activity. It was more selectively cytotoxic against cancer cells compared with normal cells, which points to the fact that grains of O. acanthium L. constitute a new plant source capable of providing useful therapeutic and preventive treatments for liver diseases and oxidative stress-related conditions. Other studies Habibatni et al., 2017; Kouki et al., 2024; Parzhanova et al., 2024(8, 25, 29) have reported Onopordum's use as a bactericide, heart tonic, hemostatic, and diuretic. It is used for nervousness, anti-tumor purposes, and inflammation of the bladder, respiratory, and urinary systems

because it contains a group of active substances such as saponins, alkaloids, sesquiterpene lactones, flavonoids, triterpenes, sterols, soluble sugars, proteins, and oils by increasing its oxidative activity.

Geographical and environmental factors have been shown to influence the chemical composition and biological activity of plants, as demonstrated by a study in Tunisia (25) on the Onopordum species. Grown in different regions of Tunisia (Sousse, Kairouan, and Nabeul), there were significant differences in phenolic composition between ecotypes and plant organs ($P < 0.05$), flowers of the Nabeul region in Tunisia showed the highest total phenolic content, while flowers grown spontaneously. Principal component analysis confirmed significant organ and genotype variation, highlighting the potential of domestication for biodiversity conservation and technological applications. According to studies(8, 30, 31) *O. acanthium* L contains flavone glycosides (quercetin, isorhamnetin, apigenin), sesquiterpenes, alkaloids, saponins, vitamin C, pigments, inulin, tannins, and flavones. ,. The group of researchers found that the content of the main fatty acids oleic, linolenic, and palmitic were also highest, as was the relationship between unsaturated and saturated fatty acids. This percentage is found in the herb Onopordum acanthium L. It is 88.5. They also calculated the amount of sterols, tocopherols, and phospholipids present in oil extracts from the plant, which confer on it the biological efficiency as an antioxidant and a traditionally used medicinal plant.

4. CONCLUSION

The current study investigates the antioxidant activity and phytochemical composition of *O. acanthium L.*, extracted from five regions in Western Iraq. Overall regional variations have been recorded, and Hit and Al-Karmah exhibited the most diversity regarding bioactive compounds that reflected the plant's adaptation. Antioxidant activity varied among regions: the highest scavenging activities were obtained for samples from Ramadi, which showed high potential for acting as antioxidants, while the lowest values were found in samples from Fallujah, likely due to unsuitable environmental conditions. The results indicate that soil type and climate are among the most significant environmental factors influencing the biosynthesis of secondary metabolites in *O. acanthium*. This work underlines the real value of this plant as a rich source of natural antioxidants for pharmaceutical and nutritional applications.

REFERENCES

[1]. Che C-T, George V, Ijinu T, Pushpangadan P, Andrae-Marobela K. Traditional medicine. Pharmacognosy: Elsevier; 2024. p. 11-28. https://doi.org/10.1016/B978-0-443-18657-8.00037-2
[2]. El Mokni R. Orobanche dominae (Orobanchaceae), a new species described from Tunisia, North Africa. Mediterranean Botany. 2023;44. https://doi.org/10.5209/mbot.80515
[3]. Kılıç Ö, Yildirimli S. Contributions to the Flora of Çelikhan District (Adıyaman). Adıyaman University Journal of Science. 2022;12(2):203-29. https://doi.org/10.37094/adyujsci.1143607
[4]. Dimeyeva L, Sitpayeva G, Ussen K, Orlovsky L, Ablaikhanov E, Islamgulova A, et al. Meadow vegetation of the zhetysu Alatau mountains. Applied ecology and environmental research. 2016;14:375-98. http://dx.doi.org/10.15666/aeer/1404_375398
[5]. Al-Snafi AE. Traditional uses of Iraqi medicinal plants (part 2). International Journal of Biological and Pharmaceutical Sciences Archive. 2021;2(1):022-41. https://doi.org/10.53771/ijbpsa.2021.2.1.0057
[6]. Mouffouk C, Mouffouk S, Mouffouk S, Haba H. Traditional use, phytochemistry and pharmacological properties of the genus onopordum. Current Chemical Biology. 2023;17(2):124-39. https://doi.org/10.2174/2212796817666230102092008
[7]. Motmainna M, Juraimi AS, Ahmad-Hamdani MS, Hasan M, Yeasmin S, Anwar MP, et al. Allelopathic potential of tropical plants—a review. Agronomy. 2023;13(8):2063. https://doi.org/10.3390/agronomy13082063
[8]. Parzhanova A, Yaneva T, Dimitrov D, Georgieva A. BIOLOGICAL POTENTIAL, HEALTH CAPACITY, AND FUNCTIONAL ACTIVITY OF EMULSION SYSTEMS OBTAINED BY INCORPORATING PLANT EXTRACTS. Journal of Hygienic Engineering & Design. 2024;46(1).
[9]. Wei C, Zhou S, Shi K, Zhang C, Shao H. Chemical profile and phytotoxic action of Onopordum acanthium essential oil. Scientific Reports. 2020;10(1):13568. https://doi.org/10.1038/s41598-020-70463-7

[10]. Berganayeva G, Kudaibergenova B, Litvinenko Y, Nazarova I, Sydykbayeva S, Vassilina G, et al. Medicinal plants of the flora of Kazakhstan used in the treatment of skin diseases. Molecules. 2023;28(10):4192. https://doi.org/10.3390/molecules28104192
[11]. Lahlou RA, Carvalho F, Pereira MJ, Lopes J, Silva LR. Overview of Ethnobotanical–Pharmacological Studies Carried Out on Medicinal Plants from the Serra da Estrela Natural Park: Focus on Their Antidiabetic Potential. Pharmaceutics. 2024;16(4):454. https://doi.org/10.3390/pharmaceutics16040454
[12]. Zenobi S, Fiorentini M, Aquilanti L, Foligni R, Mannozzi C, Mozzon M, et al. Effect of planting density in two thistle species used for vegetable rennet production in marginal Mediterranean areas. Agronomy. 2021;11(1):135. https://doi.org/10.3390/agronomy11010135
[13]. Baştürk A, Peker S. Antioxidant Capacity, Fatty Acid Profile and Volatile Components of the Onopordum Anatolicum and Onopordum Heteracanthum Species Seeds Grown in Van, Turkey. Journal of the Institute of Science and Technology. 2021;11(4):2810-22. https://doi.org/10.21597/jist.895713
[14]. Sharma OP, Bhat TK. DPPH antioxidant assay revisited. Food chemistry. 2009;113(4):1202-5. https://doi.org/10.1016/j.foodchem.2008.08.008
[15]. Yu M, Gouvinhas I, Rocha J, Barros AI. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Scientific reports. 2021;11(1):10041. https://doi.org/10.1038/s41598-021-89437-4
[16]. Li C, Gao Y, He S, Chi H-Y, Li Z-C, Zhou X-X, et al. Quantification of nanoplastic uptake in cucumber plants by pyrolysis gas chromatography/mass spectrometry. Environmental Science & Technology Letters. 2021;8(8):633-8. https://doi.org/10.1021/acs.estlett.1c00369
[17]. Al Nuaimy MAAR, Hawar SN. isolation and identification of endophytic fungi from Aloe vera leaves and chemical analysis of the alcoholic extract of the leaves using HPLC, GC, and GC-Mass devices. Ibn AL-Haitham Journal For Pure and Applied Sciences. 2024;37(1):101-17. https://doi.org/10.30526/37.1.3254
[18]. Alawode TT, Lajide L, Olaleye M, Owolabi B. Stigmasterol and β-Sitosterol: Antimicrobial Compounds in the Leaves of Icacina trichantha identified by GC–MS. Beni-Suef University Journal of Basic and Applied Sciences. 2021;10:1-8. https://doi.org/10.1186/s43088-021-00170-3
[19]. Younis MM, Ayoub IM, Mostafa NM, El Hassab MA, Eldehna WM, Al-Rashood ST, et al. GC/MS profiling, anti-collagenase, anti-elastase, anti-tyrosinase and anti-hyaluronidase activities of a Stenocarpus sinuatus leaves extract. Plants. 2022;11(7):918. https://doi.org/10.3390/plants11070918
[20]. Yamuna P, Abirami P, Vijayashalini P, Sharmila M. GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of Gomphrena decumbens Jacq. Gas. 2003:3.
[21]. Savych A, Basaraba R, Muzyka N, Ilashchuk P. Analysis of fatty acid composition content in the plant components of antidiabetic herbal mixture by GC-MS. Pharmacia. 2021;68:433-9. https://doi.org/10.3897/pharmacia.68.e66693
[22]. Al-Marzoqi AH, Hameed IH, Idan SA. Analysis of bioactive chemical components of two medicinal plants (Coriandrum sativum and Melia azedarach) leaves using gas chromatography-mass spectrometry (GC-MS). African Journal of Biotechnology. 2015;14(40):2812-30. https://doi.org/10.5897/AJB2015.14956
[23]. Angelov G, Georgieva S, Petkova-Parlapanska K. Antioxidant activity of extracts from cotton thistle (Onopordum acanthium L.). Science and Technologies. 2012;3:19-23.
[24]. Sharef AY, Hamdi BA, Alrawi RA, Ahmad HO. Onopordum acanthium L. extract attenuates pancreatic β-Cells and cardiac inflammation in streptozocin-induced diabetic rats. PLoS One. 2023;18(1):e0280464. https://doi.org/10.1371/journal.pone.0280464
[25]. Habibatni S, Zohra A, Khalida H, Anwar S, Mansi I, Awadh Ali N. In vitro antioxidant, Xanthine oxidase-inhibitory and in vivo Anti-inflammatory, analgesic, antipyretic activity of Onopordum acanthium. Int J Phytomed. 2017;9(1):92-100.
[26]. Abood SH, Mohammed MM, Mutlaq TA, Abbas HM. EFFECT OF TEUCRIUM POLIUM EXTRACT ON STAPHYLOCOCCUS AUREUS BACTERIA ISOLATED FROM WOUNDS AND BURNS. Biochemical & Cellular Archives. 2021;21(1). https://connectjournals.com/03896.2021.21.1799
[27]. Leist M, Hasiwa N, Daneshian M, Hartung T. Validation and quality control of replacement alternatives–current status and future challenges. Toxicology Research. 2012;1(1):8-22. https://doi.org/10.1039/c2tx20011b
[28]. Oueslati MA, Gunenc A, Rigane G, Ghazghazi H, Valencia C, SALEM RB, et al. Chemical composition, antioxidant and cytotoxic activities of Onopordum acanthium L. Crude oil and defatted meal. Rev Roum Chim. 2019;64(6):503-10. Doi: 10.33224/rrch.2019.64.6.06
[29]. Kouki R, Essaidi I, Annabi K, Dhen N, Haouala F, Alhudhaibi AM, et al. Chemical Composition, Antioxidant Activity, and Milk-Clotting Activity of Aqueous Extracts from Leaves, Stems, and Flowers of Three Tunisian Ecotypes of Spontaneous and Cultivated Onopordum nervosum ssp. platylepis Murb.: A Potential Novel Vegetable Rennet Option. Agronomy. 2024;14(5):987. https://doi.org/10.3390/agronomy14050987
[30]. Petkova N, Hambarlyiska I, Angelova E, Ivanov I. Fructans, polyphenols and antioxidant activity in edible roots and thistles from seven medicinal plants. CMU J Nat Sci. 2021;20(4):e2021082. https://doi.org/10.12982/CMUJNS.2021.082

[31]. Mandel JR, Dikow RB, Siniscalchi CM, Thapa R, Watson LE, Funk VA. A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. Proceedings of the National Academy of Sciences. 2019;116(28):14083-8. https://doi.org/10.1073/pnas.1903871116