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Study the effect of phenolic compounds extracted from some wild Iraqi plants as anti-leishmaniasis *in vitro*

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ABSTRACT

Background: As natural compounds, especially from plants, are considered a rich source of therapeutic importance, researchers have reported the benefits of using such products in treatments for diseases affecting humans and other organisms for many years. The study included the extraction of phenolic compounds from four genera of plants of the Compositae family growing wild in western Iraq, including: *Achilliea fragrantissima, Herba-alba Artemisia (*wormwood), *Lactuca serriola* (Wild lettuce), and *Silybum marianum*. Methodology: Hydro-alcoholic extraction was employed by the cold maceration method with 75% Ethanol. A general chemical analysis of the secondary metabolites in the crude ethanolic extract was conducted for each of the four plants separately. Thin layer chromatography (TLC) was used to estimate the types of phenolic compounds qualitatively, and the total phenols in each extracted residue were quantified using a colorimetric method with a standard curve plotted for the phenol standard (Gallic acid). The HPLC technique was also employed to identify the types and concentrations of phenolic compounds extracted from each plant residue. The effect of total phenolics extracted from each plant on cultures of the *Leishmanial* parasite was also studied in 24-hour in vitro experiments, using different concentrations of each plant's extract. The violet color was then read at the wavelength (620 nm) using an ELISA Reader device to obtain the inhibition rates, as the intensity of the color is an expression of the number of living cells. Results: The results showed that the plants contain phenols, flavonoids, tannins, glycosides, saponins, and alkaloids; however, the wormwood extract yielded a negative result for alkaloids. The results of thin-layer chromatography analysis showed that the phenolic extracts of the four plants contained, qualitatively, Pyrogallol, Cinnamic acid, gallic acid, and Hydroquinone in varying proportions. The extract also contained some types of flavonoids. Results for HPLC showed that the four plants are rich in these phenolic compounds. For quantitative total phenolic compound results, *Artemisia*, the wormwood plant, contained the highest percentage, 1464.72 mg% dry plant powder. In contrast, the lowest percentage was found in the Silybum plant, at 223.86 mg% dry plant powder. The activity against *Leishmania* growth inhibition rate showed that each plant extract exhibited a different mode of inhibiting the parasite cultures, with Silybum plant showing the best effects, and no significant difference was observed across all concentrations. These vary depending on the type of phenolic compounds and the flavonoids present in each plant, as well as their concentration. Conclusion: The four plant genera of the Compositae family growing wild in western Iraq, including: *Achilliea fragrantissima, Herba-alba Artemisia (*wormwood), *Lactuca serriola (*Wild lettuce), and *Silybum marianum*, are rich with phenolic compounds and different types of flavonoids which may help in parasite diminution, as these secondary active metabolites possess notable effects in free radical scavenging and antioxidant activity.

Key words: *Achilliea*, anti-leishmaniasis, *Artemisia*, gallic acid, *Lactuca*, *Silybum*, Wild Iraqi plants.
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INTRODUCTION

Numerous crude extracts and pure natural compounds from plants have been reported to contain many effective components, rendering them therapeutically important and making them suitable for use in treatments for diseases affecting humans and other organisms. Plants are a rich source of bioactive components, including phenolic compounds that play a crucial role in human health as antioxidants, anti-inflammatory agents, and immune system modulators, among others (1). The family Asteraceae (Compositae) is derived from the term "aster" meaning "star" in Latin, and refers to the characteristic inflorescence with flower heads composed of florets (small flowers), and surrounded by bracts, one of the most prominent families comprising 1600–1700 genera and 24,000–30,000 species(2). Most of the species are herbs and shrubs, while trees are fewer in number. Asteraceae have been commonly used in the treatment of various diseases since ancient times, as attested by classical literature (3). Members of the Asteraceae are claimed to have multiple properties: antipyretic, anti-inflammatory, detoxifying, antibacterial, wound-healing, antihemorrhagic, antalgic (also for headaches), anti-spasmodic, and anti-tussive, and have been considered beneficial for flatulence, dyspepsia, dysentery, lumbago, leucorrhoea, hemorrhoids, hypotension, and most importantly, some are hepatoprotective, antitumor and antiparasitic (4).

Plants from the *Asteraceae* family are commonly used traditionally as well as in modern medicine for the treatment of various diseases. Phenolic compounds represent one of the most widely distributed secondary metabolites in the plant kingdom, possessing biological activity responsible for many known medical benefits. Asteraceae plants rich with phenol components, such as simple phenols, flavonoids, coumarin, and others, may show an ability to inhibit parasites (5). Additionally, these compounds play a significant role in cardiovascular disorders. They possessed anti-cancer properties, which might be attributed mainly to their antioxidant activities, including scavenging free radicals, the reactive oxygen species (ROS), inhibition of peroxidation, and chelating transition metals. (6) The phenolic compounds were associated with some bioactivity, such as: antipyretic, anti-inflammatory, detoxifying, antibacterial, wound-healing, anti-hemorrhagic, antalgic (also for headaches), anti-spasmodic, and anti-tussive, and have been considered beneficial for flatulence, dyspepsia, dysentery, lumbago, leucorrhoea, hemorrhoids, hypotension, and most importantly, some are hepato-protective, antitumor and antiparasitic (7). Four plant genera belonging to the Asteraceae family were found to grow naturally as wild herbs in the Anbar lands, the western regions of Iraq, including *Artemisia herba-alba* (AR), *Achillea fragrantissima* (A), *Lactuca serriola* (L), and *Silybum marianum* (S). The larger genus is *Artemisia herba-alba*, which has high economic value in several fields, including as food plants and as anthelminthic and antimalarial agents in medicine. It is also used in both traditional and modern medicine for treating fungal and bacterial infections, as well as for its anti-diabetic properties (8). *Achillea fragrantissima* is another Asteraceae plant grown in Arabian countries as a desert plant that has been traditionally used for treating many liver and kidney disorders, wound healing, and manifestation of skin diseases, inflammations, and gastrointestinal tract disorders. As the plant contains many bioactive components with antioxidant and anti-inflammatory properties, *A. fragrantissima* could be beneficial in preventing neuroinflammation and protecting against neurodegenerative diseases, which are part of their pathophysiology, as observed in laboratory animal studies (9). The plant *Lactuca serriola* contains numerous active compounds, including vitamins, beta-carotene, iron, and oxalic acid, lactucopicrin, and sesquiterpene esters**.** Its pharmacological activity as a potent analgesic with anti-inflammatory effect is revealed to have high antioxidant phenolic contents, especially Quercetin, which shows potential free radical scavenging effects. It is traditionally used for hypnosis and sedation, as well as for cough suppression and expectorant purposes. The plant possessed antispasmodic effects and could be used as a diuretic, vaso-relaxant, demulcent, and purgative (10). Another plant that belongs to the family Asteraceae is *Silybum marianum*, commonly known as milk thistle. Milk thistle is native to Southern Europe and Asia, but it also occurs in the Canary Islands and extends eastward to Southern Russia and Iran. As the plant contains a considerable amount of

silymarin, which is regarded as a pharmaceutically interesting flavonoid-lignan mixture component, *S. marianum* has been cultivated in large fields in Austria and other regions. Additionally, this plant is rich in various flavonoids, including kaempferol, quercetin, and taxifolin, as well as fatty oils (20-30%), such as linoleic acid. Tocopherols and sterols had been identified in the fruits. The plant juice had been used for centuries in traditional medicine to stimulate bile flow and for intestinal cleansing; additionally, it appeared to be beneficial in liver disorders (11).

Annually, approximately two million people across ninety countries become infected with Leishmaniasis, which can be caused by less than 20 different species of the parasite *Leishmania*, including *Leishmania tropica* (causing cutaneous and mucocutaneous infections) *and Leishmania donovani* (causing visceral disease) (12,13). Regarding the infectious species and host immune system function, there were limited therapeutics available and effective as curative agents for leishmaniasis. In addition to the difficulty of treatment, the parasite had the ability to spread and techniques to evade the host immune system, all these reasons called for exploring various methods to target this infection and needed for new effective drugs, since the current treatment suffers from several serious problems and limitations like poor efficacy, toxicity, and resistance all that evoke and require finding alternatives leishmaniasis therapy (14).

The current project intends to study the extraction and evaluation of total phenolic contents in some wild plants to investigate the antiparasitic activity on Leishmanial species in vitro, as these plants may be rich in simple phenolic compounds, especially Gallic acid, a potent free radical scavenger, and may act as a chelating agent that will affect the growth of the parasite. Thus, the objective of the study: 1- Identify the most important simple phenolic compounds in selected plants- Estimate their quantity and quality. 3- Testing the effectiveness as an anti-leishmania parasite and comparing it with the standard substance, gallic acid (15).

METHODOLOGY

1. Plant material

The plant material was collected between March and August 2018 in the afternoon, exposed to sunlight, and then taken to the Center for Desert Studies at the University of Anbar for classification to determine the taxonomic identity of the plants, which was confirmed by the Iraqi National Herbarium. Then, the plants were dried in the shade, ground using an electric grinder, and packed into clean, sterile glass containers.

1.1. Plants Extraction (16,17)

The aerial parts of *Artemisia herba-alba*, *Achillea fragrantissima*, *Lactuca serriola*, and *Silybum marianum* were collected and powdered. An extraction procedure was employed, involving the maceration of approximately 50 g of each dried sample separately with 75% ethanol for one week in a cold, dark place with shaking. The extracts of all plants were filtered through Whatman No. 1, concentrated at 45°C using a rotary evaporator, and then each residue was stored in dry, clean containers at 4°C until use.

1.2. Phytochemical investigation (18)

General phytochemical tests were conducted to detect the presence of active components in the plant extract. These tests included: Tannins detection tests with 1% Lead acetate solution, saponins by foam formation test, Benedict test for detection of reducing sugar, Alkaloids detection by Dragendorff *test*, polyphenols test with 1% ferric chloride solution, and flavonoids detection with sodium hydroxide solution.

1.3. Total phenols Qualitative Assay by Thin Layer Chromatography (TLC)

For the estimation of the types of phenolic compounds that might be present in each plant-extracted residue, the TLC technique was employed, with different standard solutions included: Pyrogallol, Cinnamic acid, Hydroquinone, and gallic acid as simple phenol standards, and Rutin, Quercetin, Kaempferol, and luteolin as flavonoid standard solutions. A thin layer of silica-coated plate represented the stationary phase in the chromatography separation process. For the mobile phase, a mixture of chloroform, ethyl acetate, and formic acid in a ratio of 5:1:4 was used.
Each type of flavonoid or phenolic compound separated can be detected in relation to R_F value standard spots, which could be measured as follows:

$$R_F \text{ value} = \frac{\text{The distance traveled by each phenol}}{\text{Distance traveled by the mobile phase}}$$

The silica plate, after application, was exposed to UV light at 254 nm to detect colored spots (19).

1.4. Total phenols Quantitative Estimation: (20)

The Folin-Ciocalteu colorimetric method was used in this assay. Phenolic compounds, extracted from each residue, will act to transfer electrons in an alkaline medium to the Folin reagents, which contain phosphomolybdic/phosphotungstic acid, forming a colored complex that can be determined at a range of 725-765 nm with a spectrophotometer apparatus. Aliquot of 0.6 ml extract (5mg/ml) of each plant, and Gallic acid standard solutions ranging 100-20 µg/ml each in a separate tube, might be reacted with 0.5 ml Folin reagent. Then, 2 mL of Na2CO3 solution (200 mg/mL) was added to all tubes and left for 15 min at room temperature. Finally, the absorbance was measured at 760nm for all tubes. The gallic acid standard curve and the straight line equation were used for total phenols calculation in each plant residue.

1.5. Qualitative and Quantitative analysis by HPLC for the Phenolic Compounds in the four plants

The following conditions were applied according to (21) using the following standard in concentration of 5mg/ml, even for all plant extracts

Pyrogallol(P), Gallic acid(GA), Cinnamic acid(C), Hydroquinone(H), Luteolin(L), Kaempferol (K), Quercetin (Q), and Rutin (R)

Mobile phase : Methanol : 1% Formic acid (70:30).

Column: C18(250X 4.6id)mm, 5micrometer site.

Flow rate: 1 ml/min.

Injected volume: 20 microliter.

Wavelength: 254 nm.

Instrument = Shimatzu / Japan.

2. Anti-Leishmaniasis Activity

For this assay, stock solutions (1 mg/mL D.W.) from the four plants were prepared. Then, 1 mL from the stock was diluted to 4 mL with D.W., and another six dilutions were made in the same manner to obtain concentrations of (750, 562.5, 422, 316.4, 237.3, and 178) µg/mL. All solutions were sterile and filtered through a 0.22 µm Millipore filter. The assay was performed on *L. tropica* for 24 hours (22). Plates of 96 flat-bottom wells were seeded with 10^4 cells / of *L. tropica* promastigotes suspended in RPMI growth medium. Then, different sterile plant solutions in concentrations of (750, 562.5, 422, 316.4, 237.3, and 178 µg/mL) had been added in triplicate for each. The plates were incubated for 20 hours in a CO_2 incubator at 26 °C. Aliquot of 10 µL MTT dye (5 mg/mL) was added to all wells, and the plates were incubated for three to four hours. Finally, 50 µL of dimethyl sulfoxide (DMSO) was added to the formazan violet crystals for solvation. The absorbance of each well was read at 620 nm with an ELISA plate reader.

Statistical Analysis:

All data were subjected to a statistical analysis system (SAS, 2018) program to detect the differences among all factors that study parameters were included. The least significant difference (LSD) was used to compare the means significantly with ANOVA/Two-way analysis (23).

RESULTS

1.1. Ethanolic Extract Plant Residue:

The weight residues for each plant extract are shown in Table (1).

Table (1): Percentage Residue of dried ethanolic extract for aerial parts of the four plants

Plant name	Artemisia	Lactuca	Achillea	Silybum
%Residue from extraction(W/W)	24.6602	17.6746	12.9686	22.3858

1.2. Phytochemical Investigation of the Four Plants' Extracts:

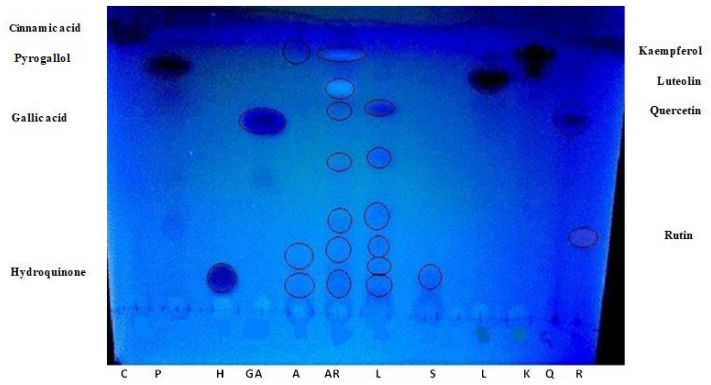
Table (2) shows the major active components detected in the extracts of the four plants.

Table (2): phytochemical tests of ethanolic plants' extract

TEST	PLANT NAME	Achillea	Artemisia	Lactuca	Silybum	Result
Detection of Tannins		+	+	+	+	White Precipitate
Detection of the saponines		+	-	+	+	Foam formation
Alkaloids Detection by Dragendorff reagent		+	+	+	+	Brown Precipitate
Flavonoids Detection		+	+	+	+	Bright yellow colour
Polyphenols Detection		+	+	+	+	Brown Precipitate
Detection of reducing sugar		+	+	+	+	Orange-Red Precipitate

1.3. Qualitative Assay for Phenolic Compounds in the fourth plants' extracts:

Figure (1) and Table (3) illustrate predicted phenolic and flavonoid compounds in the four plants extracted residue in comparison with standard phenols and flavonoids represented by their R_f values.



Figure(1): TLC Chromatogram represented; Achillea(A), Artemisia(Ar), Lactuca(L), Silybum(S), standard phenols; Cinnamic acid(C), Pyrogallol(P), Hydroquinone(H), Gallic acid(GA), and standard flavonoids; Luteolin(L), Kaempferol(K), Querecetin(Q), Rutin(R).

Table (3): R_f values for each spot in the plants' extract and standard phenols and flavonoids

Standard	Plant	Achillea	Artemisia	Lactuca	Silybum	R_f value
Cinnamic acid(C)		0.9				0.9
Pyrogallol (P)						0.83
Hydroquinone(H)		0.15	0.15	0.15	0.15	0.15
Gallic acid (GA)						0.65
Luetolin (L)						0.85
Kaempferol (K)			0.89			0.89
Querecetin (Q)			0.8	0.8		0.8
Rutin (R)			0.35	0.35		0.35

1.4. Quantitative Assay for Total Phenols:

Table(4) represented the absorption at 760 nm of different concentrations of Gallic acid standard solution, while Figure(2) illustrated the standard curve with the straight line equation obtained through total phenols in all plants extracted with the Folin-Ciocalteu reagent.

Table (4): Absorption values of different concentrations of the standard Gallic acid

Gallic Acid Standard Concentration(µg/ml)	**Absorption(760nm)**
0	0
20	0.417
40	1.764
60	1.067
80	1.241
100	1.344

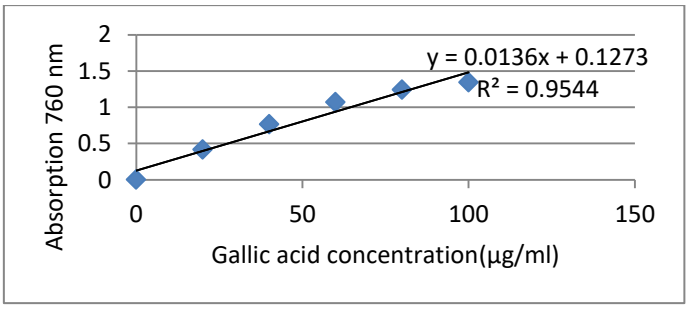


Figure (2): Standard Curve for Gallic Acid

Where Y=Absorption; for *Achillea* Y=0.978, for *Artemisia* Y=1.339, for *Lactuca* Y= 1.317, and for *Silybum* Y= 0.331

X= Concentration of total phenol (µg/ml)

From the straight line equation, Y=0.0 136 X +0.1273, when the equation was applied, each 100 g aerial part from each plant should contain total phenols as mentioned in Figure (3).

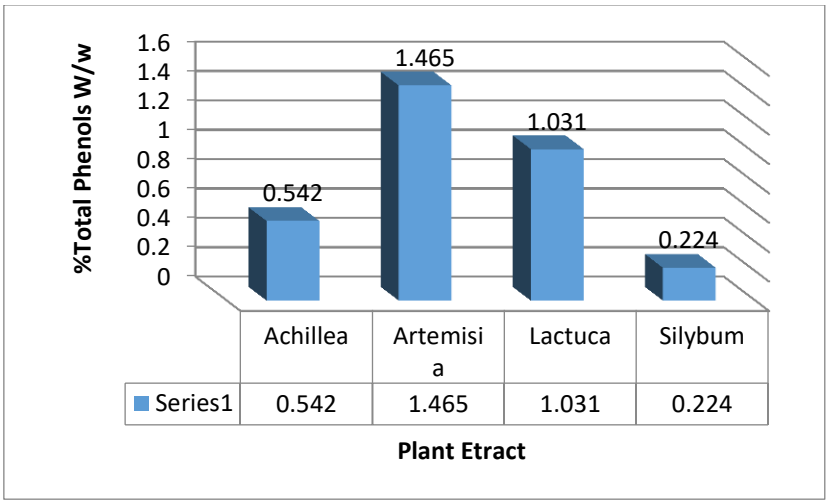


Figure (3): Total phenolic compounds as percentage W/W in each plant

1.5. Qualitative and Quantitative analysis by HPLC for the Phenolic Compounds in the four plants

The HPLC chromatogram for the standard phenols used in this work, along with their retention times in minutes, is shown in Figure (4).

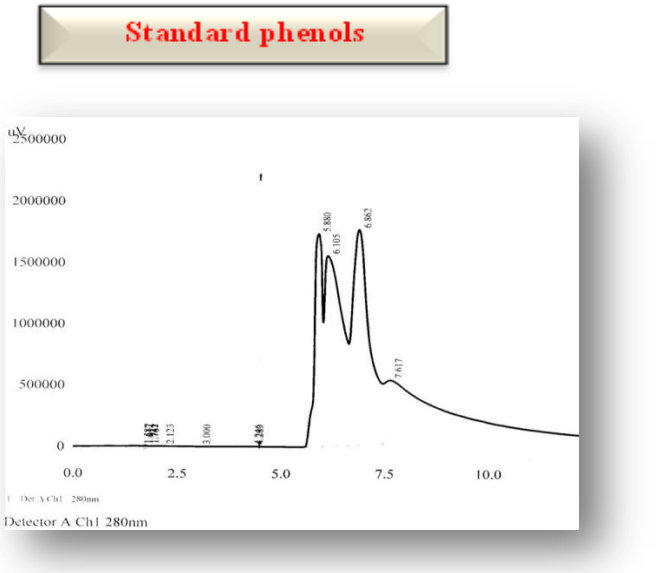


Figure (4): HPLC Chromatogram for the Standard Phenols

The phenolic compounds in the four plant extracts, *Achillea(A), Artemisia(AR), Lactuca(L),* and *Silybum(S)* were shown in figures (5), (6), (7), and (8), respectively, detected by an HPLC apparatus.

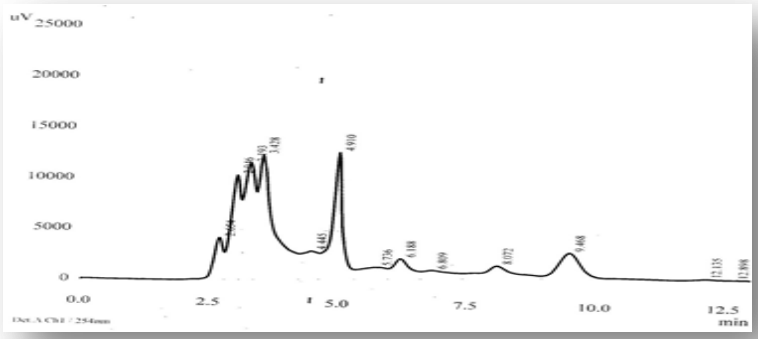


Figure (5): HPLC Chromatogram for *Achillea(A)*Phenolic Compounds

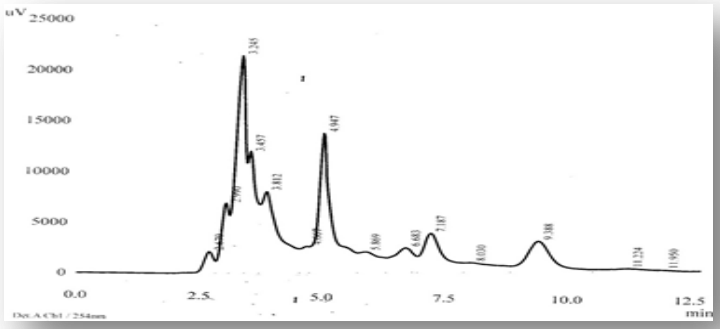


Figure (6): HPLC Chromatogram for *Artemisia (AR)*Phenolic Compounds

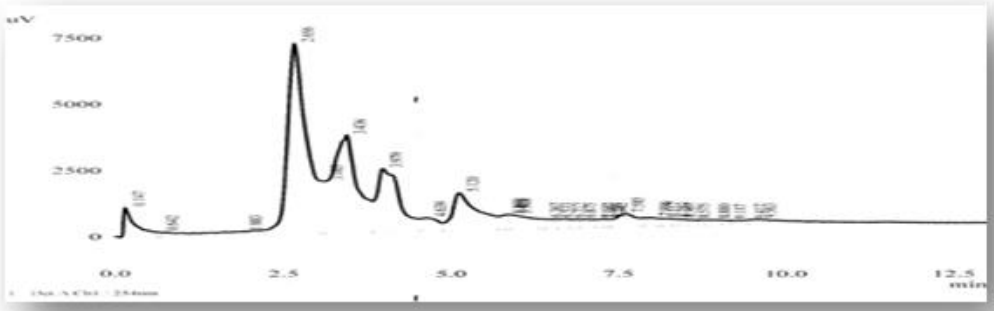


Figure (7): HPLC Chromatogram for *Laactuca(L)*Phenolic Compounds

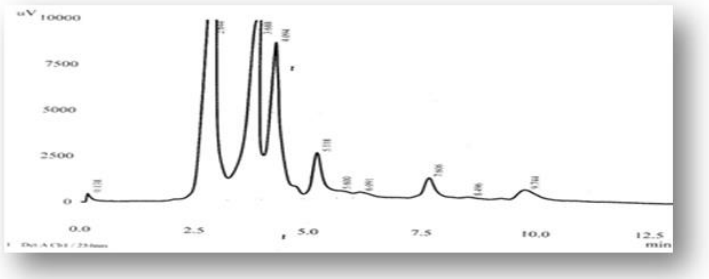


Figure (8): HPLC Chromatogram for *Silybum (S)* Phenolic Compounds

While the flavonoids retention time for the standards used in the study, along with those extracted from the four plants *Achillea(A)*, *Artemisia(AR)*, *Lactuca(L)*, and Silybum(S), were shown in Figures 9,10,11,12, respectively.

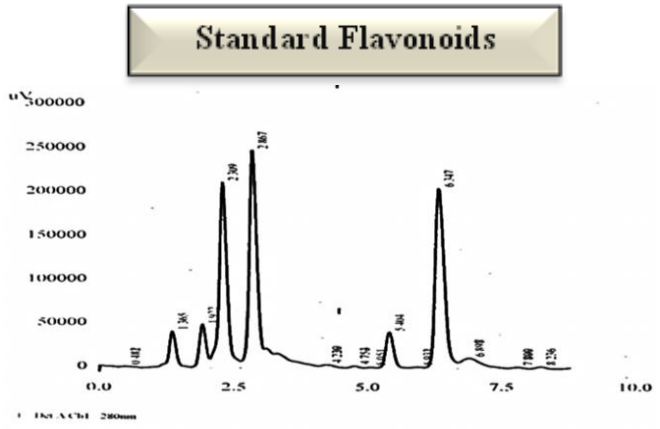


Figure (9): HPLC Chromatogram for Standard Flavonoid Compounds

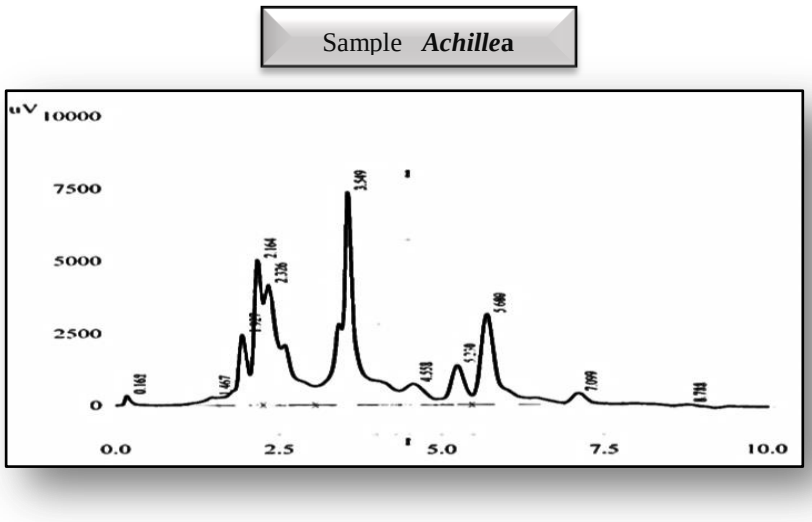


Figure (10): HPLC Chromatogram for *Achillea f. (A)* Flavonoid Compounds

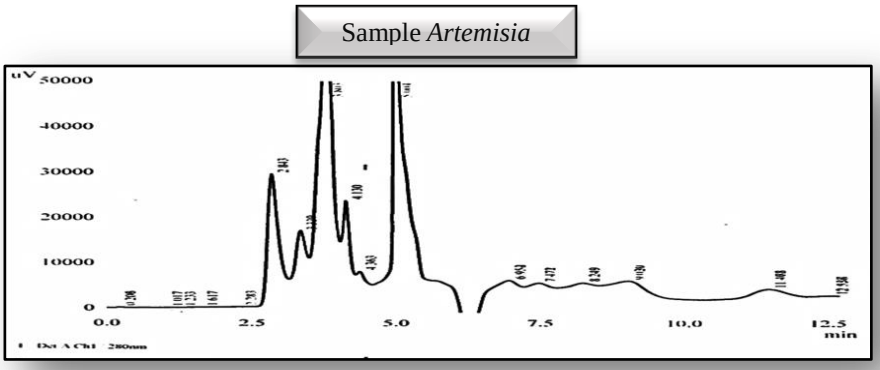


Figure (11): HPLC Chromatogram for *Artemisia (AR)* Flavonoid Compounds

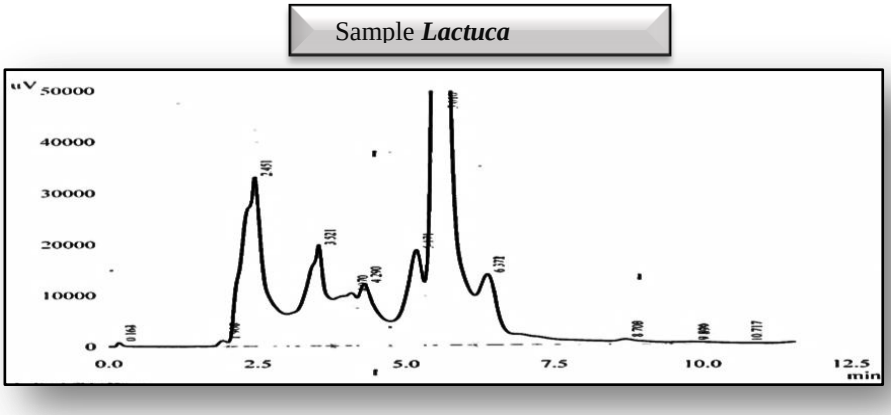


Figure (12): HPLC Chromatogram for *Lactuca (L)* Flavonoid Compounds

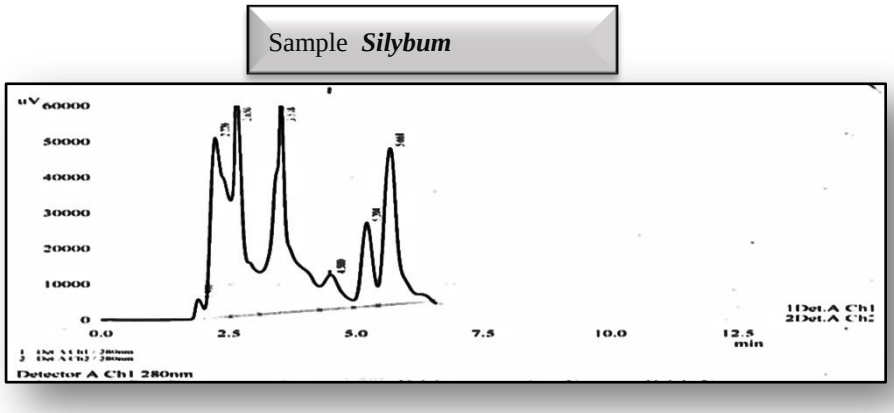


Figure (13): HPLC Chromatogram for *Silybum (S)* Flavonoid Compounds

Data employed retention time (Rt) in minutes for the standard and all plant extracted phenols and flavonoids were illustrated in Table (5).

Table (5): The retention time (Rt) in minutes for the standard and all extracted phenols and flavonoids in the four plants

Standard Phenols	**St. Rt. (min)**	**Rt(min) For The Phenolic compounds in Plants' Extracts**			
		A	***AR***	***L***	***S***
Gallic acid	5.88	5.736	5.869	5.8	**5.6**
Pyrogallol	6.105	6.188		5.908	**6.091**
Hydroquinone	6.862	6.809	6.683	6. 875	
Cinnamic acid	7.617	8.072	8.030	8.575	**8.496**
Standard Flavonoids	**St. Rt.** (min)	**Rt (min) For The Flavonoids in Plant Extracts**			
		A	***AR***	***L***	***S***
Rutin	1.922	1.927	1.617	1.906	**1.900**
Quercetin	2.309	2.326	2.283	2.451	**2.226**
Apigenin	5.404	5.230	5.004	-	**5.204**
Kaempferol	6.347	-	-	5.610	**-**
Luteolin	6.898	7.097	6.952	6.372	**-**
Coumarin	2.867	2.867	2.843	-	**2.650**

The standard and all extracted phenols and flavonoids concentrations (mg %) were shown in Table (6)

Table (6): The concentration of the standard and all extracted phenols and flavonoids in the four plants

Phenolic Compounds	**St. Conc. (mg/L)**	**Conc. mg/100g. Plant For The Phenols**			
		Af	***AR***	***L***	***S***
Gallic acid	1	-	67.8	2.5	**7**
Pyrogallol	1	102	-	8.18	**18.37**
Hydroquinone	1	53	35	12.64	-
Cinnamic acid	1	1.4	23.5	1.19	**0.025**
Flavonoids & Coumarin	St. Conc. (mg/L)	**Conc. mg/100g. Plant For The Flavonoids**			
		A	***AR***	***L***	***S***
Rutin	5	1.5	1.9	3.1	**2.8**
Quercetin	5	0.9	1.1	10	**12**
Apigenin	5	1.3	13.7	168	**21.4**
Kaempferol	5	-	-	4.3	**-**
Luteolin	5	0.5	63.9	-	**-**
Coumarin	5	-	4.9	-	**7.4**

2. Anti-Leishmaniasis Activity

Table (7): Inhibition rate percent of all concentrations for the four plants against Leishmania tropica for 24 hours

Conc. (µg/ml.)	% IR *Artemisia a.*	% IR *Achillea f.*	% IR *Lactuca s.*	% IR *Silybum m.*	L.S.D. value
750	53.75 ±2.8 A a	32.42 ±1.6 A b	22.00 ±1.2 C c	48.00 ±2.7 AB a	7.026 *
562.5	47.30 ±2.5 A b	18.13 ±0.76 C d	32.10 ±1.7 B c	50.00 ±2.8 AB a	8.471 *
422	49.70 ±2.8 A a	26.00 ±1.4 AB c	41.70 ±2.2 A b	54.00 ±3.1 AB a	7.169 *
316.4	53.25 ±3.4 A a	30.60 ±1.7 A c	39.00 ±1.7 AB b	56.50 ±3.5 A a	6.984 *
237.3	45.25 ±1.9 A ab	29.00 ±2.2 AB c	40.00 ±1.9 AB b	47.75 ±2.3 B a	7.025 *
178	31.50 ±1.6 B b	22.00 ±1.3 BC c	45.5 ±2.6 A a	49.50 ±2.8 AB a	7.317 *
0(Control)	0.00 C a	0.00 D a	0.00 D a	0.00 C a	0.00 NS
L.S.D. value	8.665 *	8.094 *	9.347 *	8.761 *	---

Means having the different big letters in the same column and the small letters in the same row differed significantly. * (P≤0.05).

Discussion

The Asteraceae family represents one of the famous plant families that are naturally grown in the western regions of Iraq, especially in the Anbar lands. The larger genera belonging to this family are the four plants included in the current study: *Artemisia herba-alba*, *Achillea fragrantissima*, *Lactuca serriola*, and *Silybum marianum*, all of which are cultivated as wild herbs in this country. Their therapeutic effects are similar to those of anthelminthic and antimalarial treatments, both traditionally and in modern times (24). Their ancient and folkloric usage in medicine may be due to the active compounds content in these plants, which were investigated in the current study (25). *Artemisia* represented the most extensive phenolic content among other Asteraceae family plants, as it has been proven to be rich in secondary metabolites, indicating major active constituents such as flavonoids and other phenols for the aerial part extraction (4,5,16). The total phenolic content as a percentage in the four plant extracts was expressed in the following descending range: *Artemisia> Lactuca> Achillea > Silybum*. Thus, these plants exhibited potent biological activity, including antioxidant, antibacterial, and hypoglycemic effects, in their various uses. Many researchers' projects have included these plants and others of this family, which have been emphasized for their curative effects in addressing significant health problems, particularly their anticancer activity, which holds promise as actual therapeutic drugs (26,27). Different phenolic compounds at varying concentration levels have been detected in the four studied plant extracts, including cinnamic acid, gallic acid, Pyrogallol, Hydroquinone, and others. Each phenolic compound type plays a distinct role in managing free radicals as an antioxidant; thus, each plant exhibits different activity as an antileishmaniasis agent.

Conclusion

The plants grown in western Iraq, especially those in the Asteraceae family, as wild, might be a natural source for several biological materials, as they are rich in essential active constituents, such as phenolic compounds. More studies should be conducted on the purification of medically and biologically important compounds, as they offer significant economic returns.

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دراسة تأثير المركبات الفينولية المستخلصة من بعض النباتات البرية العراقية كمضاد لداء الليشمانيا في المختبر

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الخلاصة

خلفية البحث: نظرًا لأهمية المركبات الطبيعية وخاصةً تلك المستمدة من النباتات، كمصدر غني لأهميتها العلاجية، فقد أفاد الباحثون لسنوات عديدة بفوائد استخدامها في علاج الأمراض التي تصيب الإنسان والكائنات الحية الأخرى. شملت الدراسة استخلاص مركبات فينولية من أربعة أجناس من نباتات الفصيلة المركبة (Compositae) التي تنمو بريًا في غرب العراق، وهي: القيصوم العطري (*Achilliea fragrantissima*)، والشيح الأبيض (*Herba-alba Artemisia*)، والخس البري (*Lactuca serriola*)، والسيليبيوم الماريائي (*Silybum marianum*). الطرق ومواد العمل: استُخدم الاستخلاص الكحولي المائي بطريقة النقع البارد باستخدام 75% من الإيثانول. وأُجري كشف كيميائي عام عن المستقلبات الثانوية للمستخلص الإيثانولي الخام لكل نبات من النباتات الأربعة على حدة. تم الكشف عن أنواع المركبات الفينولية باستخدام كروماتوغرافيا الطبقة الرقيقة (TLC)، وتقديرها نوعيًا، وحُسب إجمالي الفينولات لكل بقايا مستخلصة كتقدير كمي للمركبات الفينولية باستخدام طريقة قياس اللون، مع رسم منحنى معياري لمعيار الفينول (حمض الغاليك). كما طُبقت تقنية كروماتوغرافيا السائل عالي الأداء (HPLC) لتحديد أنواع وتركيزات المركبات الفينولية المستخلصة من كل بقايا نباتية. دُرست أيضًا تأثيرات إجمالي الفينول المستخلص من كل نبات على مزارع طفيلي الليشمانيا لمدة 24 ساعة في تجارب مخبرية، باستخدام تراكيز مختلفة من كل مستخلص نباتي. ثم قُرئ اللون البنفسجي عند الطول الموجي (620 نانومتر) باستخدام جهاز قارئ إليزا (ELISA Reader) للحصول على معدلات التثبيط، حيث أن شدة اللون هي تعبير عن عدد تلك الخلايا الحية. النتائج: أظهرت النتائج أن النباتات تحتوي على الفينولات والفلافونويدات والعفص والجليكوسيدات والصابونين والقلويدات، إلا أن مستخلص الشيح أعطى نتيجة سلبية للقلويدات. أظهرت نتائج تحليل كروماتوغرافيا الطبقة الرقيقة أن المستخلص الفينولي للنباتات الأربعة يحتوي نوعيًا على بيروغالول وحمض السينناميك وحمض الغاليك والهيدروكينون بنسب متفاوتة. كما يحتوي المستخلص أيضًا على بعض أنواع الفلافونويدات. أظهرت نتائج HPLC أن النباتات الأربعة غنية بهذه المركبات الفينولية. بالنسبة لنتائج المركبات الفينولية الكلية الكمية؛ الشيح، احتوى نبات الشيح على أعلى نسبة 1464.72 ملجم٪ من مسحوق النبات الجاف، بينما كانت أقل نسبة في نبات السيليبووم 223.86 ملجم٪ من مسحوق النبات الجاف. أظهر معدل تثبيط نمو طفيلي الليشمانيا أن كل مستخلص نباتي أظهر أسلوبًا مختلفًا في تثبيط مزارع الطفيلي، وقد أظهر نبات السيليبووم أفضل التأثيرات دون أي دلالة إحصائية في جميع التركيزات. وتختلف هذه التأثيرات باختلاف نوع المركبات الفينولية والفلافونويدات الموجودة في كل نبات، بالإضافة إلى تركيزه.

الاستنتاجات: إن الأجناس النباتية الأربعة لفصيلة Compositae التي تنمو بريًا في غرب العراق، وهي: Achilliea fragrantissima، وHerba-alba Artemisia (الشيح)، وLactuca serriola (الخس البري)، وSilybum marianum، غنية بالمركبات الفينولية وأنواع مختلفة من الفلافونويدات التي قد تساعد في تقليل عدد الطفيليات، حيث تمتلك هذه المستقلبات النشطة الثانوية تأثيرات ملحوظة في إزالة الجذور الحرة ونشاط مضادات الأكسدة.

الكلمات المفتاحية: *Achilliea*، مضاد لداء الليشمانيا، الشيح، حمض الغاليك، *Lactuca*، *Silybum*، نباتات برية عراقية.