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Immunomodulation effects of alcoholic extracts for both *Achillea* and *Artemisia* wild plants in the Iraqi desert, *In Vitro*

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ABSTRACT

Background: *Artemisia hera-alba*, *Achillea fragrantissima* are plants naturally grown as wild herbs in western regions of Iraq, especially in Anbar lands belonging to *Artemisia*, which is one of the larger genera in the family Asteraceae, and primarily used in medicine against anthelminthic and antimalarial, besides its traditional use as antibacterial, antifungal, as well as hypoglycemic effect**.** The current study aimed to highlight the critical impact of the phenolic compounds, as these plants were rich in. **Methodology**: An extraction method for *Artemisia herba-alba (AR)* and *Achillea fragrantissima (A)*, involving the processing of aerial parts through cold maceration. Both plant extracts underwent phytochemical general investigations, including qualitative and quantitative estimations, as well as antioxidant assays, to elucidate their biological activities, specifically their immune-boosting and modulatory effects. **Results**: The results indicated that the plant extract contained rich phenolic compounds, including Pyrogallol, Hydroquinone, and Cinnamic acid. Besides, the following flavonoids were present in both extracts: Rutin, Quercetin, Apigenin, Kaempferol, and Luteolin. The immune modulation effects indicated that both plant extracts showed a proliferative impact for normal human lymphocytes in higher doses, while cytotoxic effects appeared in low doses. **Conclusion**: The toxic effects of all used concentrations of both plants affected the normal viability of natural killer cells, indicating that the dose might be adjusted to avoid any toxic effects that appear in these plant extracts at different medication states.

Key words: *Artemisia hera-alba*, *Achillea fragrantissima*, Immunomodulation, phenolic compounds, Flavonoids.
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INTRODUCTION

Since ancient times until today, many raw or purified compounds from natural sources have received the attention of researchers, especially in the field of medicine and treatment, and have cured many diseases affecting humans and other organisms. (1). One of the most popular active components with medical importance in the plant kingdom is phenolic compounds. Phenolic compounds are known to be responsible for a wide range of biological activities (2). In general, they act as potent natural antioxidant components, playing a well-known role in free radical scavenging, inhibition of peroxidation, and chelation of transition metals. Consequently, these compounds exhibit anti-cancer properties and play a significant role in heart disease (1). The name of the family Asteraceae or

Compositae is derived from the term "aster" meaning "star" in Latin, and is regarded as one of the most prominent families that comprises more than 1500 genera and about 30,000 species and is distributed mainly in warm-temperate countries (3). About 7000 phenolic components were isolated from species belonging to this family that possessed potent bioactive effects against different problems, such as antibacterial, antispasmodic, antiparasitic, anti-flatulence, and dyspepsia. Besides, these components were regarded as promising agents in treating or prophylaxis of tumors and cancers (4,5). *Artemisia herba-alba* and *Achillea fragrantissima,* both of which are naturally grown as wild herbs in western Iraq, particularly in Anbar lands, belong to this family. Artemisia is one of the larger genera in the family Asteraceae, and is mainly used in medicine against anthelminthic and antimalarial, besides its traditional use as antibacterial, antifungal, and hypoglycemic effect (6). *Achillea fragrantissima* is a desert plant used in the traditional medicine of Arabian countries to treat many diseases of the liver and kidneys, diseases of the gastrointestinal tract, inflammatory, skin manifestations, or wound healing (7,8). *Artemisia fragrantissima* also has beneficial importance in pathophysiology conditions, such as in the prevention of different disorders of neurodegenerative diseases, in which neuro-inflammation is part of, and has a positive impact on Alzheimer's disease (9). Few studies have shed light on these wild plants in our country, particularly regarding their immune-modulatory effects. The present study aims to investigate the immunomodulatory effect of ethanolic extracts of each of the wild plants on normal human lymphocytes *in vitro*.

METHODOLOGY

1- Plant collection and authorization:

The plant material was collected in March from Anbar province in the western area of Iraq. Plant samples identified by the Iraqi National Herbarium confirmed all collective samples according to (Rechinger,1964) as follows

Kingdom: Plantae.
Division: Spermatophyta.
Subdivision: Angiospermae.
Class: Dicotyledones.
Order: Asterales
Family: Compositae (Asteraceae).
A-Genus: *Achillea*--- Species: *Achillea fragrantissima* (Forsk.) She.
B-Genus: *Artemisia*---Species: *Artemisia herba-alba* (Asso).

2- Extraction:

Aerial parts of the following plants, *Artemisia herba-alba and Achillea fragrantissima*, were collected, dried in shade, and then powdered. About 50 g from each sample was macerated in 75% ethanol for one week in a cold dark place, and then filtered through filter paper (Whatman No. 1). Finally, all extracts were concentrated with the aid of a rotary evaporator at 40°C. The weight of each dry plant was recorded, and then kept in a refrigerator at 40°C (10).

3- Phytochemical investigation:

The following chemical tests were performed to investigate active components in the ethanolic extract for all plants **(11):**

A. Detection of Tannins tests

A few drops of the 1% Lead acetate solution were added to the plant extract. A gelatinous or white precipitate was formed that indicated the presence of tannins.

B. Detection of reducing sugar

A liquate of 1 mL of the plant extract was mixed with 2 mL of the Benedict reagent, placed in a boiling bath for 5 minutes, and left to cool. The red deposit indicated the presence of this group.

C. Detection of alkaloids (Dragendorff test)

About 60mg of Bismuth subnitrate was dissolved in 0.2 mL of HCl (solution). Solution B contains 600mg potassium iodide in 1 mL Distell water.

The solution (A + B) was mixed and added to the plant extract; an orange to brown color will indicate the presence of alkaloids.

D. Detection of the sapponins

The detection process will proceed by shaking the solution of the plant extract well. Formation of foam at the top of the extract will indicate the presence of saponins.

E. Detection of Flavonoids

Alkaline reagent test: by using Sodium hydroxide solution, which is mixed with a small amount of plant extract solution and left, a bright yellow color is obtained in the presence of flavonoids

F. Detection of Polyphenolic Compounds

A few drops of 1% ferric chloride solution were added to the plant extract solution, and a brown deposition formed.

4- Qualitative Assay for Phenolic Compounds by Thin Layer Chromatography (TLC):

Stock solutions from each extracted plant residue were prepared by re-dissolving in Ethanol to get a solution of 0.1mg/mL. A standard phenolic solution (0.1mg/mL) was prepared, including Pyrogallol, Cinnamic acid, Hydroquinone, and Gallic acid standards. Additionally, solutions of some flavonoid standards, such as Rutin, Quercetin, Kaempferol, and luteolin, were prepared. Thin-layer chromatography (TLC) was carried out using a silica-coated silica60 plate with a thickness of 0.1 mm, which represents the stationary phase in the chromatography separation process. For the mobile phase, Chloroform (5): Ethyl acetate (4): Formic acid (1) was used.

The type of phenolic compound separated can be detected in correspondence to standard phenol spots in their distance, which is called the RF value. This value is derived from dividing the distance travelled by each flavonoid in each model phase by the distance traveled by the solvent:

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

Each phenolic or flavonoid compound can be detected separately by the exposure of the silica plate to the UV light as a colored spot. The silica plate is covered with Fluorescent material, which flashes when it binds to the active groups of phenol or flavonoids under UV at a wavelength of 254nm. The result is shown as bright spots under the UV light (12).

5- Quantitative Determination of Total Phenols:

The concentration of phenolic compounds in both extracts was determined by the Folin-Ciocalteu colorimetric method. The principal mechanism was the transfer of electrons in an alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, which are determined spectroscopically at 760 nm. Briefly, 0.6 mL of each plant extract solution in a 5mg/mL concentration was transferred to a glass tube. Then, 0.5 mL of reactive Folin-Ciocalteau reagent and approximately 2mL of Na_2CO_3 solution (200mg/mL) were added, and the mixture was shaken. The sample was then mixed with a vortex, and the reaction proceeded for 15 min. at ambient temperature. Then, distilled water was added to dilute the solution to half its original concentration, and the formed precipitate was removed by filtration. Finally, the absorbance was measured in a spectrophotometer at 760nm and compared to the Gallic acid calibration curve, which was prepared by six different concentration solutions ranging from 0 to 100 mg/mL standard Gallic acid. The same reaction was performed for all solutions (13).

6- Quantitative Assay for Phenols and Flavonoids by High Performance Liquid Chromatography (HPLC) (13):

The following conditions were applied for phenolic and flavonoid compounds present in both plant extracts.

Table (1): HPLC Conditions for Plant Phenolic and Flavonoid Compounds

Phenolic compounds	**Flavonoids**	**Phenolic acids**
Instrument	Shimadzu, Japan	Shimadzu, Japan
Samples	5mg/mL plant residue	5mg/mL plant residue
Mobile phase	A= 35% of 1% k $_2HPO_4$ (pH 4.2) B: 65%Acetonitrile	A= Methanol (70%) B= 0.1% Formic acid (30%)
Column	ODS_{C18} (250× 4.6 Id) mm, 5µm partical size	ODS_{C18} (250× 4.6 Id) mm/5µm partical size
Flow rate	0.8 mL/min	1 mL/min
Injection Volume	20 µL	20 µL
Detection wavelength	UV-Vis at λ 280nm	UV-Vis at λ 254nm
Column Temperature	Room Temperature	Room Temperature
Standards used	Rutin	Pyrogallol
	Quercetin	Gallic acid
	Luteolin	Hydroquinone
	Kaempferol	Cinnamic acid
	Apigenin	
	Coumarin	
Reference	(13)	(14)

7- Antioxidant activity for the extracted phenolic compounds from the four plants:

The antioxidant activity involved a chromophore provided by 1-diphenyl-2-picryl hydrazyl (DPPH) solution, which was affected by the plant extract, resulting in a purple color that was reduced to a yellow color, causing a decrease in absorption (13). About 250mg from each plant extract residue was re-dissolved in 50 mL of absolute methanol (5mg/mL). Then, 1 mL of the last solution was diluted up to 10 mL with methanol to get a final concentration of 0.5 mg/mL for each plant extract separately. A stock solution of standard Vitamin C had been prepared by dissolving 250mg in 25 mL methanol(10mg/mL), then serial dilutions were made (0.1,0.2, 0.3, and 0.4)mg/mL. Aliquot of 60 µL from each solution of Ascorbic acid and the prepared solution from all extracts into separate tubes. One tube contains only methanol, represented as a control. Three mL from a 0.2mM DPPH solution were added to each tube and mixed, then kept in the dark for 30 minutes. At last, all absorption at 517 nm had been read and compared with the DPPH reading.

The percent of DPPH free radical inhibition was calculated as follows:-

% Inhibition = ((Control absorbance – Test absorbance) / Control absorbance) x 100

Where the control was contained only in DPPH solution in methanol, the IC50 value (µg/mL) is the concentration required to inhibit 50% of the initial DPPH free radical, and was calculated from the graph of the inhibition curve for Ascorbic acid to compare the effect of each phenolic extract with the IC50 for ascorbic acid.

8- Immunomodulation Determination for the Plant Extract *in vitro* (15):

This Assay was conducted to investigate the immune-boosting effects of these plants on the normal human lymphatic system. The study involved preparing serial dilutions from both plant extract residue and conducting MTT assays on treated lymphocytes pooled from normal human peripheral blood samples. It also involved estimating the effects of the chosen plant concentration on the natural cellular level, isolated from humans using a specific MojoSort^{TM} human kit protocol.

a- Sample Preparations:-

To determine *in vitro* immune effects for the extracted plant residue, a sterile stock solution was prepared to obtain a concentration of 100mg/mL. The plants were serially diluted to obtain 12 concentrations (ranging from 100 to 0.049 mg/mL) and then sterilized with a 0.22 µm Millipore filter in separate sterile tubes, which were supplied for this work.

Peripheral venous blood was taken from 8healthy volunteers with an age range of 25-35 years who had never taken medication for at least 10 days. The Lymphocyte separation fluid (Lymphoprep; specific gravity=1.077g/l) was used to separate the normal lymphocytes in the general protocol for separation. The isolated lymphocyte cells were suspended in RPMI-1640 medium supplemented with 10% fetal calf serum, containing 100 units/mL penicillin, and 100µg/mL streptomycin, then transferred into an appropriate tissue culture flask and incubated for 18 hours at 37 °C in a 5% CO_2 incubator.

b- Lymphocytes proliferation Measurement by MTT Assay (15):-

Aliquots of 100 µL of suspended lymphocyte cells were seeded in each of the 96-well microtiter plates (10^4cells/well). Subsequently, 100 µL of each purified plant extract concentration was added in triplicate, with each plant extract in a separate plate. The untreated lymphocyte cells suspended in medium represented the negative control. At the end of exposure time, incubation in a CO_2 incubator at 37°C, all wells were subjected to 50 µL of MTT dye(2mg/mL), then incubated for a further 4 hours. The MTT-formazan crystals had been formed by live cells only. Formazan crystals were dissolved with100µl DMSO added to all wells, and the absorbance at 620 nm was recorded by an ELISA reader. Viable cell Lymphocytes as a percentage was calculated as follows:

(Absorbance of the test /Absorbance of negative control)X 100.

A comparison between the results of both plant extracts at different concentrations was statistically calculated to choose the most effective dosages of each extract that may cause lymphocytes proliferation, which can be used in further experiments as an immune-modulator.

c- Estimating the effects of chosen plant concentrations on the level of natural killer cells isolated by the specific MojoSort^{TM} human kit protocol. (16,17).

Two final concentrations from each plant extract residue had been used in this assay: 12.5mg/mL and 0.625 mg/mL. The exact steps for lymphocyte cells isolation, as in the MTT assay, were performed using two tissue culture plates of 24 wells, one for each plant. Aliquots of 500 µL of suspended lymphocyte cells (approximately 1 × 10^7 cells/100 µL) were seeded in each of the 24-well tissue culture plates. Subsequently, 500 µL of each plant extract concentration was added in triplicate, with each plant extract in a separate plate. The untreated lymphocyte

cells suspended in medium represented the negative control. The plates were incubated for 3 hours in a CO_2 incubator at 37 °C. Each well was pooled in a separate vacuumed glass tube at the end of the exposure time. Isolation of natural killer cells from each treated and untreated sample was done using a specific kit protocol (MojoSortTM human kit protocol), catalog #.480053; San Diego, which pursued this purpose. The principle of this kit depended on magnetic particles to separate the unwanted cells from NK cells in the sample. The lymphocyte cells were ready, which were isolated from healthy human peripheral blood by depletion of non-target cells (negative selection), and finally suspended in supplemented RPMI 1640 culture medium. Cell number and viability were determined using trypan blue staining. The protocol steps were as follows: The cells were resuspended in 4 mL MojoSort™ Buffer in a 5 mL (12 x 75 mm) polypropylene tube. The cells were centrifuged at 2000 rpm for 5 minutes and resuspended in an appropriate volume of MojoSort™ Buffer. The cell concentration was counted to 1 x 10^6 cells/mL. Biotin-Antibody Cocktail 10µl was added to 100 µL of cell suspension, then mixed and incubated on ice for 15 minutes. The cells were washed by adding MojoSort™ Buffer up to 4mL, and then centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and resuspended in 100µl of MojoSort™ Buffer. The beads were mixed by vortex, and then Streptavidin Nanobeads (10µl) were added and incubated on ice for 15 minutes. The cells were washed by adding MojoSort™ Buffer up to 4mL, and then centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, and the cells were resuspended with 2.5mL of MojoSort™ Buffer. The tube was placed in the magnet for 5 minutes. The unlabeled fraction was poured out and then counted using a hemocytometer chamber.

RESULTS

1-Plant Extract residue

Table (2) indicates the amount of residue that was yielded upon ethanolic extraction of the two Asteraceae plants used in this study.

Table (2): Residue of dried ethanolic extract for 50g aerial parts of plants from total phenol extraction

Plant name	***Artemisia***	***Achillea***
Residue from extraction(g/50g)	12.3301	6.4842

2-Phytochemical Investigation of the Plants' Ethanolic Extract:

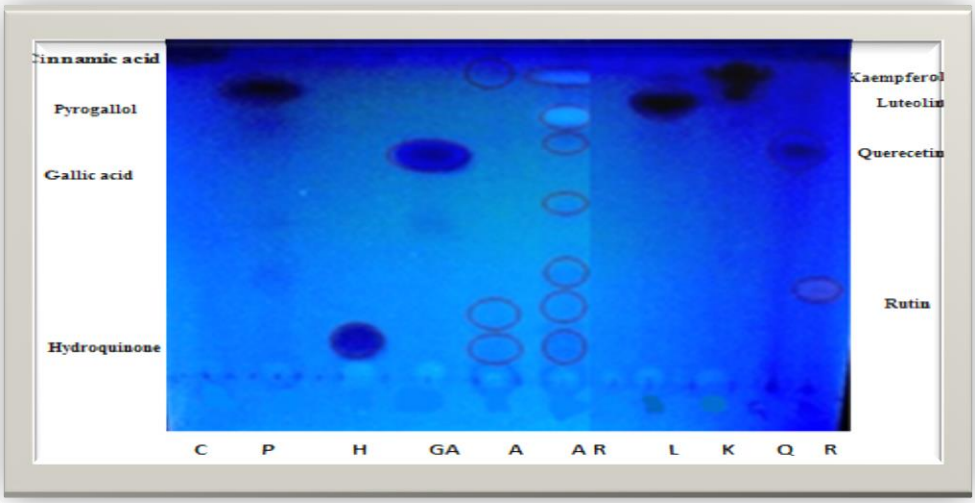
A major active constituent were detected in both plants' extracts, shown in Table (3).

Table (3): Phytochemical tests of the ethanolic plant extract

TEST	**PLANT NAME**	***Achillea* (A)**	***Artemisia* (AR)**	**COMMENTS**
Detection of Tannins		+	+	White p.p.t.
Detection of reducing sugar		+	+	Orange-Red p.p.t.
Detection of alkaloids (Dragangroff)		+	+	brown p.p.t.
Detection of the saponines		+	-	Foam formation
Detection of Flavonoids		+	+	Bright yellow
Detection of Polyphenolic Compounds		+	+	Brown p.p.t

3- Qualitative Assay for Phenol Compounds by TLC

Many phenolic compounds and flavonoids were detected in the four plant extracts, as shown in Figure (1). The chromatogram indicates the presence of different types of phenols and some flavonoids in different plant extracts, which can be detected in comparison with standard phenols and flavonoids represented by Rf values, as shown in Table (4).



Figure(1): TLC Chromatogram *Achillea*(A), *Artemisia*(AR), and in corresponding to standard phenols; Cinnamic acid(C), Pyrogallol(P), Hydroquinone(H), Gallic acid(GA), and standard flavonoids; Luteolin(L), Kaempferol(K), Quercetin(Q), Rutin(R).

Table (4): R_f values for each spot in plant extract and standard phenols and flavonoids

Standard	Plant	R_f value	*Achillea*	*Artemisia*
Cinnamic acid(C)		0.9	0.9	--
Pyrogallol (P)		0.83	--	--
Hydroquinone(H)		0.15	0.15	0.15, 0.17
Gallic acid (GA)		0.65	--	--
Luetolin (L)		0.85	--	--
Kaempferol (K)		0.89	--	0.89
Querecetin (Q)		0.8	--	0.8
Rutin (R)		0.35	--	0.35

4-Total Phenols Quantitative Assay

The amount of total phenols found in the aerial part of each plant were estimated using the visible spectrometer at 510 nm depending on absorption of the different concentrations for the standard Gallic acid curve, Table(5) where the straight line equation is obtained as shown in Figure(2), after the chemical reaction with folin- ciocalteu reagent.

Table (5): Absorption values of different concentrations of the standard Gallic acid

Gallic Acid Standard Concentration(mg/mL)	Absorption(760nm)
0	0
20	0.417
40	1.764
60	1.067
80	1.241
100	1.344
Achillea fragrantissima (2.5mg Residue/mL)	0.978
Artemisia herba-alba (2.5mg Residue/mL)	1.339

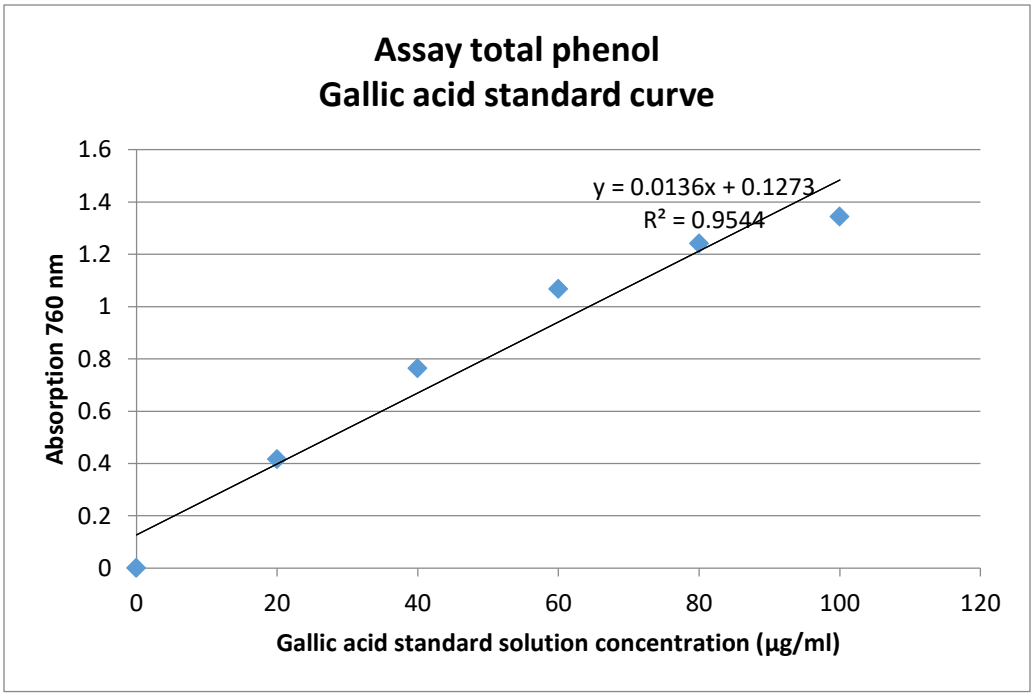


Figure (2): Gallic acid standard curve

From the straight line equation:
y= 0.0 136x +0.1273; Y=Absorption; X=Concentration of total phenol (mg/mL)
Each 100 g of aerial part from each plant will contain total phenols that can be calculated as in Table (6).

Table (6): Absorption value and percentage of total phenolic compounds in each plant

Plant Extract	Total Phenol(g/100g plant)
Achillea	0.542
Artemisia	1.465

5- Qualitative Assay for Total Phenols by HPLC:

The following figures represent the standard phenolic chromatogram.

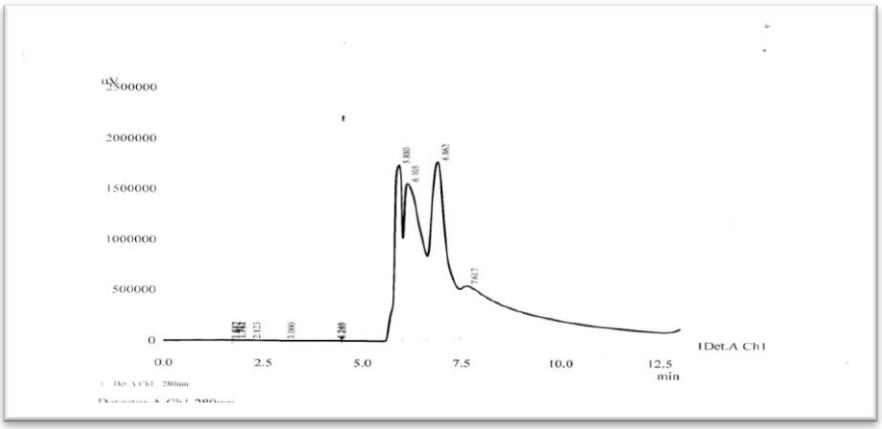


Figure (3): HPLC Chromatogram for Standards phenols

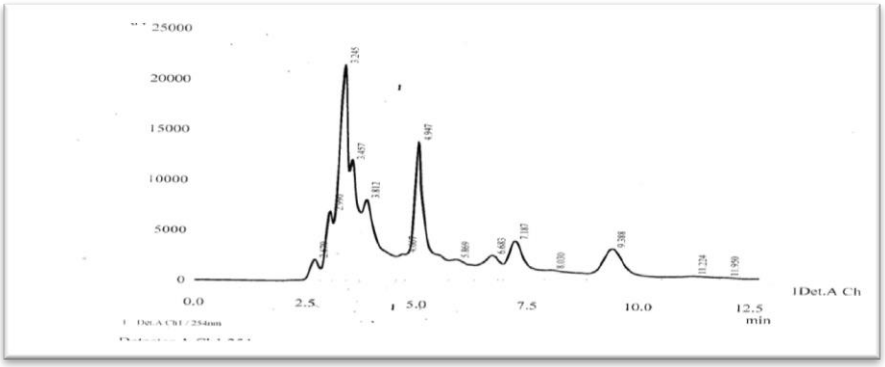


Figure (4): HPLC Chromatogram for AR phenols

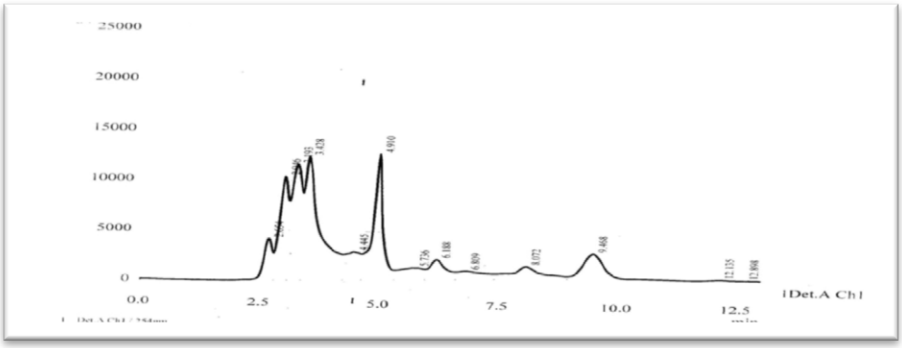


Figure (5): HPLC Chromatogram for *Achellia* phenols

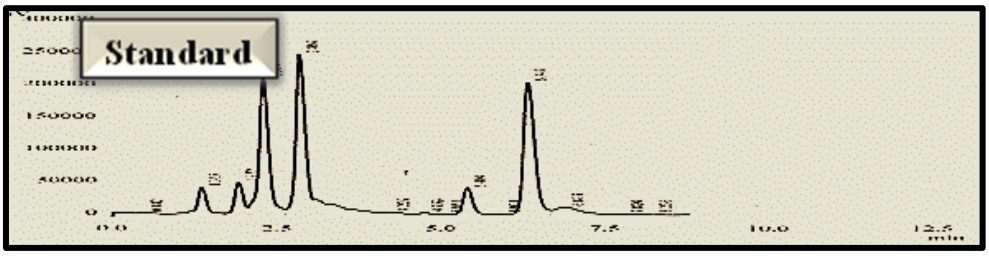


Figure (6): HPLC Chromatogram for Standard Flavonoids

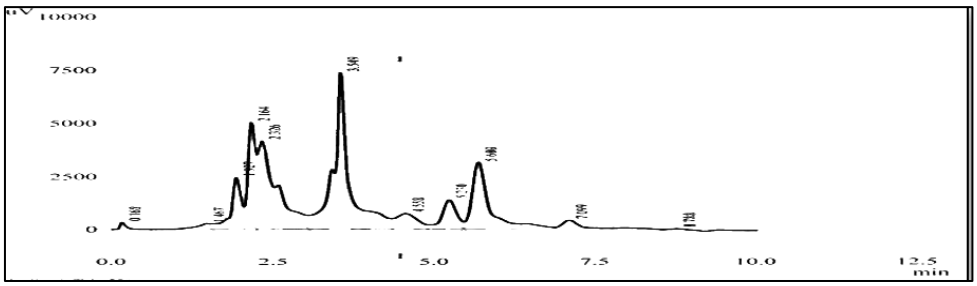


Figure (7): HPLC Chromatogram for *Achillea fragrantissima*(A)Extracted Flavonoids

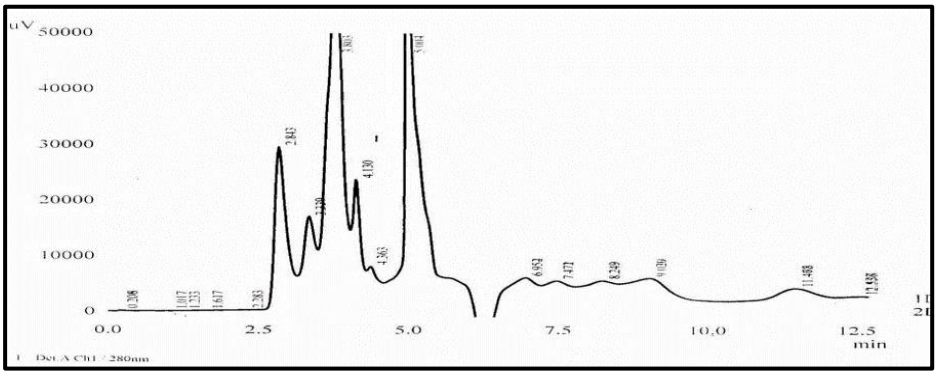


Figure (8): HPLC Chromatogram for *Artemisia herba-alb*(AR) Extracted Flavonoids

Table (7): HPLC Retention time (minutes) for Standards and the plant extract phenolic and flavonoid compounds

Standard Phenols	**Rt.(min) Standard**	**Rt.(min) A. Extract**	**Rt.(min) AR. Extract**
Pyrogallol	5.880	5.736	5.869
Gallic acid	6.105	6.188	-
Hydroquinone	6.862	6.809	6.683
Cinnamic acid	7.617	8.072	8.030
Standard Flavonoids	**Rt.(min) Standard**	**Rt.(min) A. Extract**	**Rt.(min) AR. Extract**
Rutin	1.922	1.927	1.917
Quercetin	2.309	2.326	2.333
Apigenin	5.404	5.230	5.004
Kaempferol	6.347		
Luteolin	6.898	7.097	6.952
Coumarin	2.867		2.843

6- Antioxidant Activity for the extracted phenolic compounds from the two plants:

Table (8) shows the antioxidant activity of ascorbic acid at different concentrations

Table (8) % Inhibition (free radical inhibition toward DPPH) as antioxidant activity of ascorbic acid at different concentrations

Ascorbic acid concentration(mg/mL)	Antioxidant activity(%Free radical inhibition)
0.1	3.130
0.2	5.391
0.3	57.826
0.4	67.043

From plotting the inhibition capacity against ascorbic acid concentration, the IC_{50} of Ascorbic acid can be calculated from the straight line equation, which was equal to 0.318 mg/mL, as shown in Figure (9).

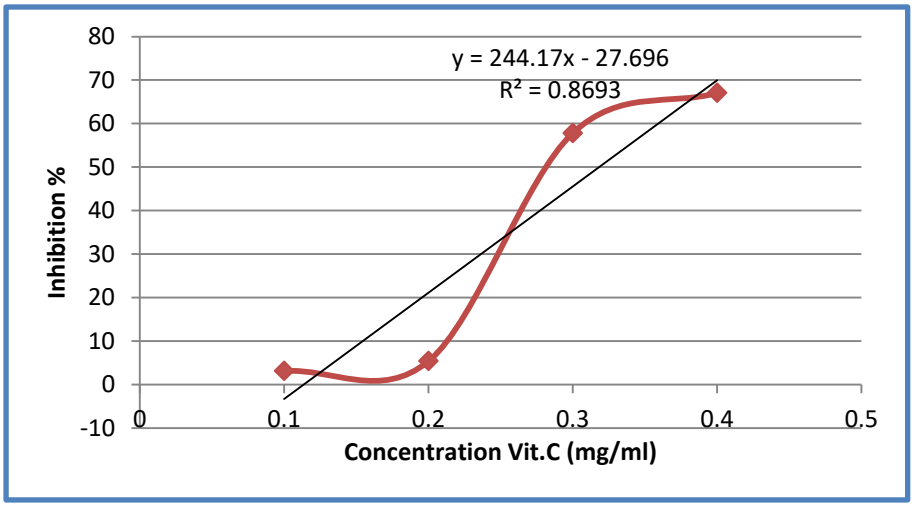


Figure (9): % Inhibition of different Ascorbic acid concentrations on DPPH free radical

The antioxidant activity of each extracted phenolic compound from the corresponding four plants is shown in Table (9).

Table (9): %Antioxidant activity of the two plants

Plant name	Concentration mg/mL	%Antioxidant activity
Achillea fragrantissima(A)	0.5	19.913
Artemisia herba-alb(AR)	0.5	58.521

7- Immunomodulation Determination for the Plant Extract *in vitro*:

a- MTT Assay

The following Figure (10) indicates the effects of different concentrations of both plant extracts on normal human peripheral lymphocytes culture.

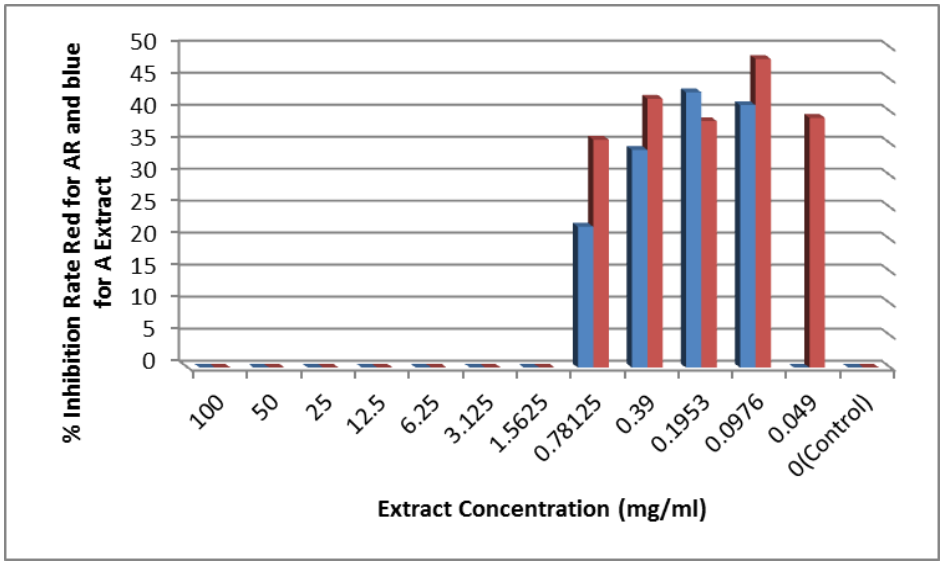


Figure (10): Histogram for the percent inhibition rate for the two plants, *Achillea fragrantissima*(A), blue column, and *Artemisia herba-alb*(AR), red column, at different concentrations on normal human peripheral lymphocytes culture

B- Estimating the effects of chosen plant concentration on the nature cell level isolated by the specific MojoSort™ human kit protocol.

The viability of NK cells (Table 10) is presented as mean ± SE. For non-treated cells, the percentage was 100% at 24 hours of incubation. The rate of viability for these cells had been affected by both plant extracts at the two concentrations in different ways. There was a decrease at all concentrations, but the major effect appeared when the normal lymphocytes had been treated with ***Achillea fragrantissima* (A)** extract at a concentration of 12.5 mg/mL.

Table (10): Effect of different concentrations of *Achillea fragrantissima* (A) and *Artemisia herba-alb* (AR) extracts on viability of NK after 24 hours of exposure time. Concentration (mg/mL)

Plant Extr. / Concentration(mg/mL)	Mean ± SE of Viability (%)
A1(12.5)	30
A2(0.625)	75
AR1(12.5)	40
AR2(0.625)	60
Untreated(control)	100

Discussion

Wild plants in western Iraq, particularly those of the Asteraceae family, are rich in fundamental active constituents, specifically phenolic secondary metabolites. More studies should be conducted to purify these compounds due to their significant biological effects.The differences in antioxidant activity between phenolic compounds extracted from each plant may depend on the types and levels of these phenols, which have shown good DPPH radical inhibition results. The most significant amount of residue from the aerial part of the ethanolic plant extract was yielded by Artemisia. The more phenolic compounds in a plant, the more biological activity (13). Thus, this plant is a rich source with many active constituents that provide insight into the use of ancient plants as medical plants, even in folk and traditional medicine (14). Many studies on the Asteraceae family have indicated that the major active constituents are represented in phenolic compounds, especially flavonoids and other simple phenols (14,15,18). The correlation between phenolic compounds and the antioxidant capacity, as measured by the DPPH method, revealed differences in the antioxidant values of the extracted phenolic compounds between the two plants. This showed that *Artemisia herba-alba* exhibited a more potent antioxidant capacity, as measured by this method. These results agree with many studies, including those on the phenolic compound antioxidant activity in such plants (19, 20, 21). All these reasons have made these plants and other species of this family of genuine interest to researchers' projects, emphasizing their anticancer activity as promising drugs (22).NK cells are essential elements in the immune defense against pathogens and tumor cells by modulating both innate and adaptive immune responses. They are directly cytotoxic to the tumor-derived and virus-infected cells. Many strategies are investigated to enhance NK cell activity in order to combat tumor growth. Results presented show that the NK cell activity of peripheral blood can be induced to varying levels by some plant extracts *in vitro*. A significant and consistent pattern of enhancement was observed by (23), who demonstrated the mechanisms of the flavonoid on NK-cell-mediated toxicity, which might be due to their favored expression of perforin and granzyme cytolytic proteins as well as up-regulation of various cytokines.

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تأثير المستخلصات الكحولية على تعديل المناعة لنباتي الأخيليا والشيح البريين في صحراء العراق (في المختبر)

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الملخص

خلفية البحث: الشيح الأبيض (*Artemisia hera-alba*) والاخيليا العطرية (*Achilea fragrantissima*) نباتان يُزرعان طبيعيًا كأعشاب برية في المناطق الغربية من العراق، وخاصةً في أراضي الأنبار، وينتميان إلى عائلة الشيح. الشيح هو أحد أكبر أجناس عائلة النجمية، ويُستخدم غالبًا في الطب ضد طاردات الديدان ومضادات الملاريا، إلى جانب استخدامه التقليدي كمضاد للبكتيريا والفطريات، بالإضافة إلى تأثيره الخافض لسكر الدم. **الهدف من الدراسة:** وفي الدراسة الحالية هدفت إلى إبراز التأثيرات المهمة للمركبات الفينولية حيث أن هذه النباتات غنية بها. **المواد وطرق العمل**: تم إجراء طرق استخلاص للأجزاء الهوائية من الشيح الأبيض (AR) والاخيليا العطرية (*Achilea fragrantissima*) باستخدام طرق النقع البارد. خضعت بقايا مستخلص كلا النباتين لدراسات كيميائية نباتية عامة، وتقديرات نوعية وكمية، بالإضافة إلى اختبار مضادات الأكسدة لشرح أنشطتهما البيولوجية في تعزيز المناعة وتعديلها. **النتائج:** أشارت النتائج إلى أن كلا المستخلصين النباتيين غنيان بالمركبات الفينولية مثل؛ بيروغالول، هيدروكينون، وحمض سينماميك. إلى جانب الفلافونويدات، وُجدت في كلا المستخلصين: روتين، كيرسيتين، أبيجينين، كامبفيرول، ولوتيولين. أشارت تأثيرات تعديل المناعة إلى أن كلا المستخلصين النباتيين أظهرا تأثيرات تكاثرية على الخلايا الليمفاوية البشرية الطبيعية بجرعات عالية، بينما ظهرت تأثيرات سامة للخلايا بجرعات منخفضة. **الاستنتاج**: أثرت التأثيرات السامة لجميع التركيزات المستخدمة من كلا النباتين على قابلية الخلايا القاتلة الطبيعية للحياة، مما يشير إلى إمكانية تعديل الجرعة لتجنب أي تأثير سام ظهر على هذه المستخلصات النباتية في حالات دوائية مختلفة.

الكلمات المفتاحية: الشيح الأبيض، الأخيلية العطرية، تعديل المناعة، المركبات الفينولية، الفلافونويدات.