Phytochemical investigation and antioxidant activity of total phenols in the aerial parts of some Asteraceae family wild plants grown in western of Iraq

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ABSTRACT

In this study Phenolic compounds of aerial parts from four wild plants belongs to Asteraceae family that grown in Anbar land located western of Iraq was employed. These are: Achellia, Artemisia, Wild lettuce (Lactuca), and Silypum .Extraction was carried on by the method of cold maceration with 75% Ethanol. Qualitative estimation was done using thin layer chromatography technique (TLC) to detect types of simple phenols that these plants rich with. While total phenols of all these plants were estimated quantitatively through Gallic acid standard curve applied from Folin-ciocalteu colorimetric reaction for total phenols estimation. An investigation for the antioxidant activity using DPPH free radical scavenger was employed in this study. Qualitative results indicated that these plants may contained some important phenolic compounds such as Pyrogallole, cinammic acid, gallic acid and Hydroquinone in different range in each of the corresponding plant

INTRODUCTION

Many effective components in crud or purified plant extracts had been reported to possess therapeutic importance, as they have been used for several years in treat and cure many diseases affecting humans and other organisms. [1].

One of the most popular active compounds possessed medical importance in the plant kingdom is phenolic compounds. Phenolic compounds are known to be responsible for many biological activities [2]. In general, they act as potent natural antioxidant components as they play important role in free radical scavenging, and inhibition of peroxidation and chelating transition metals, thus these compounds showed anti-cancer properties and play important role in heart disease [1].

The name of family Asteraceae or Compositae is drived from the term "aster" meaning "star" in Latin, and regarded as one of the largest families that comprising more than 1500 genera and about 30,000 species [3], and distributed mostly in warmtemperate countries. About7000 phenolic components were isolated that had potent bioactivity in GIT problems as antispasmodic, antiantiflatulence and dyspepsia .Also these compounds act as antibacterial,antiparasitic and good prophylaxes or treating tumors and cancers [4].

Artemisia hera-alba, Achillea fragrantissima, Lactuca serriola and Silybum marianum; are four plants naturally grown as a wild herbs in western regions of Iraq especially Anbar lands belongs to this family. Artemisia is one of the larger genera in the family Asteraceae, and mostly used in medicine against anthelminthic and antimalarial beside its tradition use as antibacterial, antifungal as well as hypoglycemic effect [5].

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. It has antioxidant and anti-inflammatory effects. The bioactive compounds isolated from A. fragrantissima may be beneficial in the prevention of neurodegenerative diseases in which included in this study. Quantitatively; the result concluded that wild Artemisia possess the major content of total phenols reach to about 1.5% of plant dried leaves. The less phenolic content was about 0.224% found in wild Silypum. The antioxidant activity for the phenolic extract of the four plants was showed that Wild lettuce (Lactuca) extract possessed the potent activity, while the lowest activity was showed by wild Silypum.

Keywords: Achellia, Artemisia, gallic acid, Lactuca, and Silypum.

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neuroinflammation is part of a pathophysiology and have a positive impact on the course of Alzheimer's disease, but they are only studies in laboratory animals [6].

Lactuca serriola : The plant traditionally was used in several medicinal conditions as anti-sedative, anti-hypnotic, cough suppressant, moreover used as purgative, demulcent, diuretic, antiseptic, also used as antispasmodic, this because that the plant contains a lot of active components, such as many vitamins, and minerals, moreover; lactucopicrin, and sesquiterpene esters. All These pharmacological activities is due to high total phenolic contents like the flavonoid "Quercetin" that showed efficient free radical scavenging potential [7].

Silybum marianum also belongs to the family Asteraceae, also known as milk thistle, and because the plant contains the largest amount of the pharmaceutically active compound knowen as silymarin (3-6%), in addition, to other flavonoids like quercetin, kaempferol, taxifolin as well as fatty oils (20-30%), the plant was cultivated in different rejoin in the world. The plant also rich with linoleic acid, tocopherols and sterols that have been accumulate in the fruits. Traditionally; the plant has been for the treatment of hepatic disorders, also the plant's juice stimulating the bile flow and intestinal cleansing [8]

The present study aimed to identify the most important phenolic compounds by TLC technique in these four types of plants that naturally grown as wild herbs in western of Iraq and estimate total phenolic compound in each plant quantitatively.

MATERIAL AND METHODS

Plant material

The plant material were collected from March up to mid-June 2018 from Anbar country at western area of Iraq. All samples identified by the Iraqi nation herbarium to confirmed all collective samples.

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Extraction

Aerial parts for the following plants: Artemisia hera-alba, Achillea fragrantissima, Lactuca serriola and Silybum marianum were collected and dried under shade, then powdered. About 50 g from each sample was macerated in 75% ethanol, for one week in cold dark place, then to be filtered through filter paper (Whatman No. 1), Finally all extracts were concentrated with aid of rotary evaporator at 40°C. The weight of each dry plant were recorded, then kept in refrigerator at 4C0 [9].

Phytochemical investigation

The following chemical tests were proceeded to investigate active components in the ethanolic extract for all plants[10]:

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 polysaccharides

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

C. Detection of alkaloids (Dragangroff test)

About 60mg of Bismuth subnitrate were dissolved in 0.2ml 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 be proceeding by shaking the solution of the plant extract well. Formation of foam at the top of the extract will indicate presence of sapponins.

E. Detection of Flavonoids

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

F. Detection of Polyphenolic compounds

Few drops of 1% ferric chloride solution were added to the plant extract solution a brown deposition will formed.

Qualitative Assay for Total phenols by Thin Layer Chromatography (TLC)

A stock solutions from each total phenol extracted of each plant were prepared by re-dissolving (5) mg Residue in10 ml of 75% ethanol to get a stock solution 0.5mg/ml. A standard phenolic solution (0.5mg/ml) also prepared including:Pyrogallol, Cinnamic acid Hydroqinon and Gallic acid standard and some flavonoids standard like Rutin, Quercetin, Kaempferol, and luteolin solutions were prepared also. 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 and for the mobile phase: Chloroform(5): Ethyl acetate(4): Formic acid(1) was used.

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

The distance traveled by each phenol

value=_

Rf

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 [11].

Quantitative Determination of Total phenols:

The concentration of phenolic compounds in the extracts was determined by the Folin-Ciocalteu colori-metric method. The assay relies on the transfer of electrons in alkaline medium phenolic from compounds to phosphomolybdic/phosphotungstic acid complexes, which are determined spectroscopically at adsorption range between (725-765 nm). Briefly; 0.6 ml of each plant extract solution in 5mg/ml concentration was transferred to glass tube, then 0.5 ml of reactive Folin-Ciocalteau reagent, and about 2ml of Na2CO3 solution (200mg/ml) were added and shaken. The sample was then mixed with vortex and reaction proceeded for 15 min. at ambient temperature. Then distilled water was added to dilute the solution half started concentration, and the formed precipitate was removed by filtration. Finally the absorbance was measured in a spectrophotometer at 760nm and compared to Galic acid calibration curve which was prepared by six different concentration solutions range from 0 up to100mg/ml standard Gallic acid proceeded the same reaction for all solutions [12].

Antioxidant activity for the extracted phenolic compounds from the four plants

This method is to determine antioxidant activity rapidly which involves a radical chromophore, simulating the reactive oxygen species, and the free radical included in: 1-diphenyl-2picryl hydrazyl (DPPH) solution that possess purple color. When plant extracts were added to DPPH solution, phenolic compounds acted as an antioxidant or a radical species scavenger, the DPPH is reduced to DPPH-H (yellow color), that causes decrease of the absorption[13]. The Procedure included the following steps.

i- Preparation of plant phenolic extracts:

about 250mg from each phenolic plant extract was redissolved in 50 ml 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 (0.5mg/ml) for each plant extract separately.

ii- Preparation of Ascorbic acid (Vitamin C) standard solutions:

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A stock solution of standard Vit.C by dissolving 250mg in 25 ml methanol(10mg/ml), then the following dilution was made (0.1,0.2, 0.3, and 0.4)mglml.

iii- Aliquot of 60 μ l from each solution of Ascorbic acid and the prepared solution from all extract each in a separated tube with another tube contain only methanol as control.

iv- Three ml from 0.2mM DPPH solution were added to each tube and mixwd well by vortex.

v- All tubes were kept in dark for 30 minutes. Ay last all tubes were read with spectrophotometer apparatus at 517 nm. All reading was compared with the DPPH reading.

vi- The percent of DPPH free radical inhibition was calculate as follow:-

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

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

RESULTS

Total Phenols From Ethanolic Plants Extract:

Table(1)indicate the amount of residue that yielded upon ethanolic extraction of the four Astereacea plant used in this study

Table 1. Residue of dried ethanolic extract for 50g	erial parts of plants from total phenol extraction
0	1 1 1

Plant name		Artemisia	Lactuca	Achillea	Silybum
Residue extraction(g/50g)	from	12.3301	8.8373	6.4842	11.1929

The largest amount of residue from aerial part of the ethanolic plant extract yielded by Artemisa. The more phenolic compounds content in a plant, the more biological activity[13].

Phytochemical Investigation of the Plants Ethanolic Extract:

A major active constituents were detected in the four plants extract, As shown in Table(2) these plants are rich with many active constituents that give an explanation about ancient plants usage as medical plants even in folk and traditional medicine[14].

Table 2. results of Phytochemical tests of ethanolic plants extract

TEST	PLANT NAME	Achillea	Artemisia	Lactuca	Silybum	COMMENTS
Detection	of Tannins	+	+	+	+	White p.p.t.
Detection Detection polysaccha	of Tunnins of arides	+	+	+	+	Orange-Red p.p.t.
Detection (Dragangr	of alkaloids off	+	+	+	+	brown p.p.t.
Detection saponines	of the	+	-	+	+	Foam formation
Detection	of Flavonoids	+	+	+	+	Bright yellow
Detection Polypheno compound	of lic ls	+	+	+	+	Brown p.p.t

Total Phenols Qualitative Assay

Many phenolic compounds and flavonoids were detected in the four plants extracts as shown in figure(1), The chromatogram indicates the presence of different types of phenols and some flavonoids in different plant extract which can be detected in comparison with standard phenols and flavonoids represented by Rf values as shown in Table(3). Noora Ibrahim issa et al: Phytochemical investigation and antioxidant activity of total phenols in the aerial parts of some Asteraceae family wild plants grown in western of Iraq



Figure 1) TIC ("hormstores debilled(b) detenticie(b) Lecture(L) ("hormstores and in corresponding to standard phenols" Figure 1. TLC Chromatogram Achillea(A), Artemisia(Ar), Lactuca(L), Silybum(S) and in corresponding to standard phenols; Cinnamic acid(C), Pyrogallol(P), Hydroquinone(H), Gallic acid(GA), and standard flavonoids;Luteolin(L), Kaempferol(K), Querecetin(Q), Rutin(R).

Standard	Plant	R _f value	Achillea	Artemisia	Lactuca	Silybum
Cinnamic aci	id(C)	0.9	0.9			
Pyrogallol	(P)	0.83				
Hydroquinor	ne(H)	0.15	0.15	0.15, 0.17	0.15 +others	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 +others	

Table 3. Rf values for each spots in plants extract and standard phenols and flavonoids

Many studies included Asteraceae family indicated that the major active constituents represented in phenolic compounds specially flavonoids and other simple phenols [5,15,16].

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(4) where the straight line equation is obtained as shown in Figure(2), after the chemical reaction with folin- ciocalteu reagent.

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	

 Table 4. Absorption values of different concentrations of the standard Gallic acid





Figure 2. Gallic acid standard curve From straight line equation y= 0.0 136x +0.1273; Y=Absorption; X=Concentration of total phenol (mg/ml) Each 100gm aerial part from each plant will contain total phenols can calculated as in Table(5).

Plant Extract(2.5mg/ml)	Absorption (760nm)	(W/W)Total Phenol/100g plant
Achillea	0.978	0.542
Artemisia	1.339	1.465
Lactuca	1.317	1.031
Silybum	0.331	0.224

As shown in Table(5), the aerial parts of Artemisia was very rich in total phenolic content, thus the plant possessed potent biological activity as antioxidant, antibacterial as well as in folic uses as a hypoglycemic herb. All these reason made the plant and the others of this family in researchers' projects to emphasize their anticancer activity as actual promise drugs [17].

Antioxidant Activity for the extracted phenolic compounds from the four plants:

Table (6) showed the antioxidant activity of ascorbic acid at different concentrations

Table 6. % 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 figure, the IC50 of Ascorbic acid can be calculated from the straight line equation which was equal to 0.318 mg/ml as showed in figure (3)



Figure 3. % Inhibition of different Ascorbic acid concentrations to DPPH free radical The antioxidant activity of each extracted phenolic compounds from the corresponding four plants was showed in Table (7)

Plant name	Concentration mg/ml	%Antioxidant activity
Achillea fragrantissima	0.5	19.913
Artemisia herba-alb	0.5	58.521
Lactuca serriola	0.5	66.347
Silybum marianum	0.5	3.826

A fact can explain the differences between phenolic compounds with their antioxidant activity extracted from each plant; may depend on the types and the level of these phenols which showed the good DPPH radical inhibition results. The mechanism of extract antioxidant activity done by DPPH free radical scavenging activity, is that the DPPH radical when receiving an electron from antioxidant compound would be reduced to stable DPPH derivative and the violet color of DPPH radical turned into yellow [18]. This correlation between phenolic compounds and the antioxidant capacity by DPPH method causes differences in the antioxidant values of the extracted phenolic compound for the four the plants. Results showed that the potent antioxidant capacity measured by this method was by Lactica phenolic extract, then Artemisia herba-alba and the lowest activity was in Silybum extract. These results agree with many studies including the phenolic compound antioxidant activity in such plants[19, 20, 21].

CONCLUSION

Wild plants grown in western of Iraq specially Asteraceae family rich with very important active constituents that is the phenolic secondary metabolites. More studies should be done for purification of these compounds as their great biological effects.

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