Evaluation of antioxidant activity, phenolic, flavonoid and ascorbic acid contents of three edible plants from Erbil/Kurdistan

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Abstract
This study was designed to examine the *in vitro* antioxidant activity of methanol extracts of *Malva sylvestris, Gundelitournefortii andEminium jaegeri* and to determine their total contents of phenolics, flavonoid and ascorbic acid. The extract were screened for their possible antioxidant potentials by using *in vitro* –antioxidant models including total antioxidant capacity, reducing power and metal chelating activity . The total contents of phenol, flavonoid and alkaloid, ascorbic acid were quantitatively estimated in edible part of these plants which is including leaves, stem and root. Results showed that the methanol extracts of these plants exhibited significant antioxidant activity by different assays and contained significant levels of phenolics, flavonoids and ascorbic acid.The methanolic extract of *Eminim jaegeri* exhibited higher antioxidant, reducing power, and metal chelating activity than the methanolic extracts of other plants and has highest phenolic, flavonoid, alkaloid and ascorbic acid content. *Gundeli tournefortii* has shown similar activity whereas the methanolic extract of *Malva sylvestris* exhibited the lowest activity.Overall result showed that these plants can serve as good source of bioactive polyphenols in the human diet and can be regarded as good candidates for nutritional supplement formulation due to their high concentration of total phenol compounds, flavonoid, ascorbic acids as well as their strong antioxidant activity.

Keywords: *Malva Sylvertris,Gundeli Tournefortii , Eminium Jaegeri,* poly phenols, flavonoids, ascorbic acid.antioxidant

Introduction
Reactive oxygen species (ROS) generated in a situation of oxidative stress .ROS play an important role in pathogenesis and pathophysiology of a variety of diseases including cancer, inflammatory disorders, atherosclerosis, carcinogenesis, drug toxic, reperfusion and neurodegenerative disease[1].The oxidation process is one of the most important roles for producing free radicals in foods, drug and even living systems [2]. Antioxidant can play a preventive role for the above mention diseases. Human body has multiple mechanism and antioxidant systems which protect the cellular molecules against damage induced by free radical [3]. However, generally these systems don't exercise sufficient protection against oxidative stress. Hence certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidant in order that the reactive oxygen species (ROS) in human body are balanced. Several studied have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease [4]. There are two basic categories of antioxidants, namely synthetics and natural , in general , synthetics antioxidants are compounds with phenolic structures of various degree of alkyl substitution , whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), or carotenoids as well as ascorbic acids [5]. Synthetics antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) have been used as antioxidant since the beginning of this century. Restriction on the use of these compounds, however, is being imposed because of their carcinogenicity [6]. Thus an interest has increased considerably [7]. Recent research investigations have suggested that diet rich in polyphenolic compounds and flavonoids are associated with longer life expectancy [8]. Moreover, these compounds have been found effective in many health-related properties such as anticancer, antiviral, anti-inflammatory activities, effect on capillary fragility and an ability to inhibit human platelet aggregation [9].

Kurdistan/ northern Iraq has a great of plant genetic resources, some of which are wild edible plants, they are used for different purposes as natural food. Wild edible plant species appear in the early spring and are consumed as vegetable sources.

In particular, despite the widespread use of wild edible plants as food in northern Iraq, the literature contains few reports on the antioxidants activity and chemical composition of these plants.

The aim of this study is to examine the antioxidant activity of methanolic extract of *Malva Sylvestris, Gundelia Tournefortii,* and *Eminium jaergeri* using the phosphomolybdenum method, reducing power, metal chelating, and vitamin C content. Another aim was to evaluate whether the total phenolic, flavonoid and alkaloid contents of wild edible plants were correlated with antioxidant activity in *vitro* may be of value in design of further studied to scavenges novel treatment strategies of disorders associated with ROS induces tissue damage.

Materials and Methods
Collection of plant materials.
Malva Sylvestris, Gundelia Tournefortii, and Eminium jaergeri were collected from local market Erbil.

Extraction of plant material

Each plant was air dried in the dark, and grounded into fine powder. The powdered materials (20.0g) were extracted with 200 ml of absolute methanol for 24 h at room temperature. The suspensions were then filtered and air-dried. The extracts were stored at -20 °C until being used [10],[11]. Designation of the individual plants and their origin are given in Table1 and Fig 1.

Chemicals

Ferrous Chloride, Aluminum chloride, Folin-Ciocalteu's phenol reagent, sodium carbonate, gallic acid, rutin, Ferrozine, ascorbic acid were purchased from Sigma-Aldrich Chemical Co. potassium ferricyanide, sodium nitrite, TCA, Sodium phosphate, ammonium molybdate, EDTA, Ferric chloride, 1,10 – phenanthroline, Colchicine , were purchased from Merck chemical Supplier (Germany. All other chemicals and reagents were of analytical grade and obtained from across.

Table 1: Names of the wild edible plants traditionally consumed and used in Kurdistan/ Iraq

Plant species	Family	Local name	Parts used	Methods of using
Malva sylvestris L	Malvaceae	Tolka	leaf	Roasting with egg
Gundelia Tournefortii, L	Asteraceae	Kangir	flower, Steam	Roasting with egg, soap
Eminium jaergeri L	Araceae	Kardii	leaf	Roasting with rice, soap

Fig 1:

Determination of the total phenolic compounds

The total phenolic compound contents were determined using the Folin-Ciocalteu reagent [12]. 0.1 ml extract was mixed with 1.0 ml of Folin-Ciocalteu's reagent; 4.0 ml of 1.0M $NaCO_3$ was added and incubated in a water bath for 2.0 hour. The total phenols were determined by spectrophotometer at 760 nm. The calibration curve was prepared with gallic acid solution ranging from (2.5 -12.5 µg/ml), The concentration of total phenolic compounds in the extract was determined as µg of gallic acid equivalent using as equation obtained from the standard gallic acid curve expressed as µg gallic acid /g dry weight of the plant material . The data were presented as the average of three analyses.

Determination of the total flavonoids

The total flavonoid contents of sample plants were determined according to the colorimetric method [13]. Briefly, 3.0 ml solution of extract was mixed with 8.0 ml dd H_2O, 0.75 ml of (5%) sodium nitrite, 0.75 ml of (10%) aluminum chloride , 5.0 ml of 1.0 M sodium hydroxide and then volume completed to 25 ml with dd H_2O, left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 515 nm. Rutin was chosen as a standard. Using the standard curve (10 -60 µg/ml), the level of the total flavonoid contents in sample extract were determined in triplicate, respectively. The results were calculated into µg rutin equivalents/g dried plant materials.

Determination of total alkaloids

The total alkaloids contents in the extract were measured using 1, 10-phenanthroline methods described by [14]. 1.0 ml of extract was mixed with 1.0 ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1.0 ml of 0.05 M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70ºC. The absorbance of red colored complex was measured at 510 nm against reagent blank. Alkaloid contents estimated and it was calculated with the help of standard curve of Colchicine in a range (20-200 µg/ml). The values were expressed as µg Colchicine equivalents/ g dried plant materials.

Determination of ascorbic acid

Ascorbic acid was determined using the method described by [15]. The metabolic extract was diluted with 10 ml of 0.5% oxalic acid and the mixture was shaken for 45 min on orbital shaker at 2000 rpm at room temperature and filtered through Whatman No.4 filter. Precisely 1.0 ml of the filtrate was mixed with 9.0 ml of 0.1mol L^{-1} of 2, 6-dichlorophenolindophenol. The volume was completed to 25ml and absorbance was read within 30 min at 515 nm against the prepared blank. The ascorbic acid content (20-100 µg/ml) was calculated using calibration curve, prepared from L-ascorbic acid.

Determination of total antioxidant activity

The total antioxidant capacity of the extract was evaluated by the phospho molybdenum method according to the procedure described by [16].The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate /Mo (V) complex at acid pH. Volume of extract contain (25-100µg/ml) was mixed with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank dd water was used in place of extract. The tube containing the reaction solution were capped and incubated in a boiling water bath at 95 ºC for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer. The antioxidant capacity of extracts was compared with standard ascorbic acid in a range (25-100µg/ml).

Determination of reducing power

The reducing power of the extract was determined according to the method of Oyaizu [17].2.5 ml of the sample (20-100 µg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, PH 6.6) and potassium ferric cyanide (2.5ml, 1.0%).The mixture was incubated at 50ºC for 20 min. A portion (2.5 ml, 10%) of tri chloro acetic acid was added to the mixture to stop the reaction, and then centrifuged at 1000 ×g for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as standard for compared with samples. Phosphate buffer (PH 6.6) was used as blank solution.

Metal chelating activity

The chelating of ferrous ions by the extract was estimated by the method of [18].1.0 ml of sample (4.0-20 µg/ml) was added to a solution of 2.0 mM $FeSO_4.7H_2O$ (0.05 ml). The reaction was initiated by the addition of 5.0 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured at 562 nm. The percentage of inhibition of ferrozine $-Fe^{+2}$ complex formations was calculated by the given formula.

Metal chelating effect (%) = $[(A_0-A_1)/A_0\times100]$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the extract or standards. Na_2 EDTA was used as standard in a range (0.0-20 µg/ml). The control contains $FeSO_4.7H_2O$ and ferrozine.

Results and Discussion

Several biochemical assays were used to screen the antioxidant properties: total antioxidant capacity (phosphomolybdenum method), reducing power, metal chelating activity, poly phenol, flavonoid, alkaloid and ascorbic acid content. The assay performed in methanolic extract of the plants. Crude extract in the plants are responsible for their bioactive properties.

Total phenol, flavonoid, alkaloid and ascorbic acid

The total phenolic compounds, total flavonoids, total alkaloid and ascorbic acid were measured for all samples. The result is given in Table 2. There was a wide range of phenolic concentrations in selected edible plants. Significant amount of three plant species ranged from 537.1 to 341 µg GAE/g. *Eminiumjaergeri L* exhibited highest amount of phenolic wherease the lowest total phenol capacity was shown with *Malva* sylvestris *L*. The methanolic extract of the plants with high amount of phenolics exhibited strong antioxidant activity. Phenolic compounds are found in edible and inedible plants and they have multiple biological effects, including antioxidant activity [19].The high potential of phenolic to scavenge free radical may be due to many phenolic they possess[20].

Total flavonoid value of methanolic extracts of the plant ranged from 85.5 to 50.10 µg rutin/g dry plant. The high value was observed in methanolic extract of *Eminiumjaergeri L* whereas the lowest value was shown with *Malvasylvestris L*. Flavoninod are very important plant constituents because of active hydroxyl group and show antioxidant activity [21].The aromatic rings of the flavonoid molecue allow the donation and acceptance of electron from free radical species [22].

The content of total alkaloids value of methanolic extract of edible plant ranged from 153.72 to 61.37 µg colchicines /g dry plant. The highest value was observed with *Eminiumjaergeri L* whereas *Malvasylvestris L* was shown the lowest value. It is to be expected that several activities might be related to a possible action from alkaloids [23]. The extract of the edible plants were most abundant in phenolic and alkaloid which contribute to the total phenolic levels.

Ascorbic acid acting as a chain breaking process of formation of antioxidant impairs with formation of free radical in the process of formation intraocular substance throughout the body, including collagen, bone matrix and tooth dentine [24].The quantitive determination of ascorbic acid in methanol extract of these plant shows that they are good source of ascorbic acids. High quantity of ascorbic acid was found to be in *Eminiumjaergeri L* whereas in *Malvasylvestris L* was recorded to have the least value of 17.64 A striking pathological change resulting from ascorbic acid deficiency is the weaking of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substance. Therefore, a clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pain in the join can be related to the association of ascorbic acid and normal connective tissue metabolism. [25].

Table 2: Total phenol, flavonoid, alkaloid and ascorbic acid contents of the three wild edible plants

Vitamin C content mg/g	Total alkaloid content µg/g	Total flavonoid content µg/g	Total phenol content µg/g	Extract
18.64	153.72±0.121	85.5±0.0487	537.10±0.0596	*Eminiumjaergeri L* (Kardii)
17.85	112.90±0.137	57.65±0.042	428.01±0.0075	Gundelia Tournefortii, L (Kangir)
17.64	61.37±0.107	50.10±0.07	341.10±0.0134	*Malvasylvestris L* (Tolka)

Total antioxidant activity

The phospho molybdenum method usually detects antioxidant such as ascorbic acid, some phenolics, α-tocopherol and carotenoids [16]. Ascorbic acid, glutathione, poly phenol and aromatic amines have the ability to donate hydrogen and electrons and that can be detected by the three assay models. The antioxidant activity of methanolic extract of wild edible plants presented in Fig 2.Total antioxidant capacity of the extracts, expressed as equivalents of ascorbic acid (µg/g of dry material). The result below was shown that the antioxidant activity of the methanolic extract of these wild edible plants were increasing with increases their concentration. The result presented below also indicated that the antioxidant activity of these plants has to be due to the presence of poly phenols, flavonoid that may act by donating electrons and free radicals. The antioxidant breaks the free radical chain by donating a hydrogen atom [26].

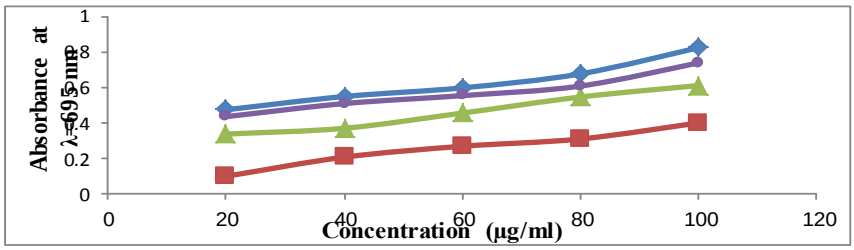

Fig (2): Antioxidant (♦) for Ascorbic acid, (●) for *Eminium jaergeri L* (Kardii), (▲) for *Gundelia Tournefortii L* (Kangir) and (■) for *Malva sylvestrisL* (Tolka)

Reducing power assay.

Another assay that is the mechanism related to electron denoting ability of the extract is reducing power assy. In this assay the presence of electron donor in the sample would result in reduction of Fe^{+3} /ferricyanide complex to Fe^{+2}.Then the amount of Fe^{+2} complex can be measuring by the formation of Perl's Prussian blue at 700 nm.For the determination of reducing power activity, Fe^{+3} to Fe $^{+2}$ reduction. The methanol extract of these plants showed significant reducing power activity. An increasing in the reactive ability. (Fig 3), shows the dose- response curves for the reduction power of the extract. It was found that reducing power of the extract also increase with increasing of its concentration. Vitamin C was used as standard. Similar relation between Fe^{+2} reducing activity and total phenol contents have been reported in the literature [27].

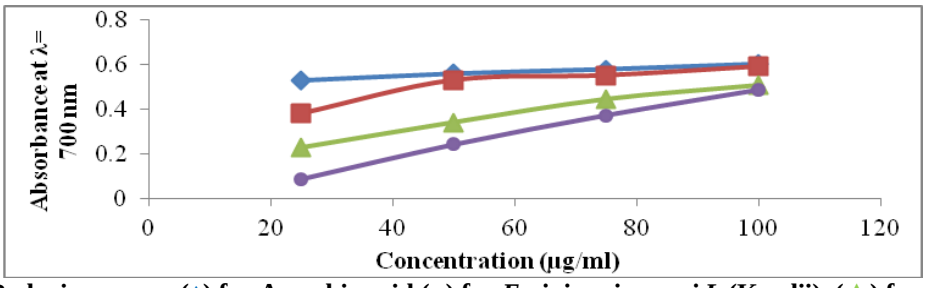

Fig (3): Reducing power (♦) for Ascorbic acid (■) for *Eminium jaergeri L* (Kardii), (▲) for *Gundelia Tournefortii L* (Kangir) and (●) for *Malva sylvestrisL* (Tolka).

Metal Chelating activity assay.

Ferrous iron can initiate lipid peroxidation by Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxayl and alkoxyl radical [2]. Chelating agent may inhibit radical generation by stabilizing transition metals, consequently reducing free radical damage. Inaddation some phenolic compounds exhibit antioxidant activity through the chelating of metal ions [28]. Ferrozine can make complexes with ferrous ions. In the presence of chelating agents complex(red color) formation is interrupted and as a result, the red color of the complex is decrease .Phenol compounds may be permit that bond to metal ions due to their chemical structure . In this assay, the methanol extract of wild edible plants and standard antioxidants interfered with formation of a ferrous-ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine, that can be shown (in Fig 4). Chelating agents that form σ bonds with a metal are effective as secondary antioxidant because they reduce the reduction potential, and there by stabilize the oxidized form of the metal ion[29]. .This study shows that these edible plants have a marked capacity for iron binding, suggesting the presence of poly phenol that has potent iron chelating capacity. The antioxidant activities of phenolic compounds were attributed to its redox properties, which allow them to act as reducing agents and have metal chelating properties [30].

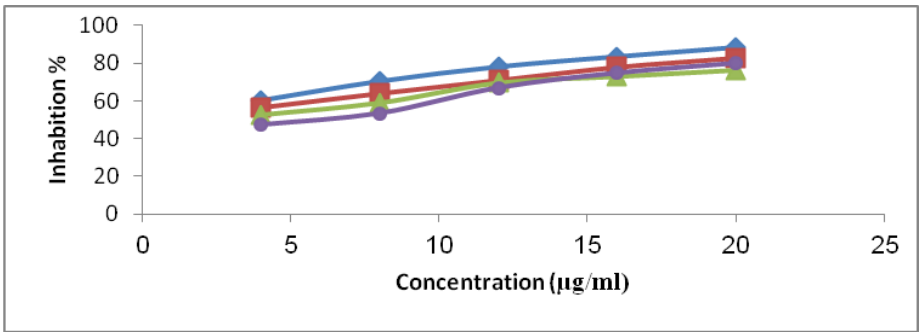

Fig (4): Ferrous ion chelating activity (♦) for Na $_2$EDTA, (■) for *Eminium jaergeri L* (Kardii), (▲) for *Gundelia Tournefortii L* (Kangir) and (●) for *Malva sylvestrisL* (Tolka)

Conclusion

The result obtained in the present study indicates that methanolic extract of this plant exhibited a wide variety of antioxidant, phenolic content. The overall antioxidant activity of this wild plant might be attributed to its poly phenolic content .Thereby; it can say that the phenolic compound of the plants is very important for antioxidant activities. The antioxidant potentials of phenolic contents of these plants could provide chemical basis for some areas of food industries, health benefits, medicine and pharmacology. Therefore, food consumption through wild edible plants is likely to me more important and effective than nutritional supplement for the primary prevention of acute diseases. Hence this result could be used as a new source for literature. As far as our literature survey could ascertain.

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تقييم محتويات انشطة مضادات الأكسده ، الفينولات ،الفلافونويدو حامض الاسكوربيك من ثلاثة نباتات صالحة للأكل في اربيل/كردستان

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الملخص

صممت هذه الدراسة لفحص نشاط مضادات الأكسدة من المستخلص الميثانولي لخبيز، الكعوب والصميعه وتحديد محتوياتها من فلافانويد الكلي، الفينولات وحامض الاسكوربيك. تم عرض نماذج لمضادات الأكسدة لمستخلص الميثانولي للنباتات الصالحة للأكل تضمنت قياس نشاط كفاءة مضادات الأكسدة الكلية ، القدرة المختزلة و الفعالية المخلبيه للفلز. وقدرت كمية محتويات مجموعها لفينول والفلافونويد والقلويد و حامض الاسكوربيك في الجزء الصالح للأكل من هذه النباتات والتي تتضمن الأوراق والساق والجذر .وأظهرت النتيجة أنا لمستخلص الميثانولي من هذه النباتات لها نشاطا ملحوظ اللأكسدة بواسطة فحوصات مختلفة، و كذلك احتوت على مستويات كبيرة من الفينول و فلافانويد وحمض الاسكوربيك . احتوى المستخلص الميثانولي من الصميعه أعلى مضاد للأكسدة، القدرة المختزله و الفعاليه المخلبيه للفلز مقارنة بالمستخلص الميثانولي من غيرها من النباتات المذكورة أعلا هو كذلك كانت لها اعلى الفينولات ، فلافونويد، قلويد و محتوى حامض الاسكوربيك .وقد أظهرت الدراسة ان الكعوب لها نشاط مماثل في حين أن المستخلص الميثانولي من الخبيزيه عرضت أدنى نشاط .

يستنتج من نتائج هذه الدراسة ان هذه النباتات يمكن أن تكون بمثابة مصدر جيد لمادة البولي فينول ذات التأثير الأحيائي في النظام الغذائي للإنسان، ويمكن اعتبارها مرشحا جيد الصياغة المكملات الغذائية بسبب احتوائها على تراكيز عالية من مركبات الفينول الكلي، الفلافونويد وحامض الاسكوربيك و كذلك نشاطها القوي لمضاد للأكسدة.