Total Phenolics, Antioxidant Activity and Anti-Diabetic Capacities of Selected Iraqi Medicinal Plants

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Abstract

Introduction: In Iraq, medicinal plants have not received much attention in terms of quantifying their antioxidant and other biological/pharmacological activities so the objective of the present study was to assess the antioxidant activity of selected medicinal plants grown in Iraq and relate it to their total phenolic contents and their inhibitory effect on carbohydrate-hydrolyzing enzymes.

Methods: Antioxidant activity was assessed by ferric reducing antioxidant power (FRAP) and oxygen reactive absorbance capacity (ORAC) methods. The possible inhibitory effect on carbohydrate-hydrolyzing enzymes was also investigated.

Results: In almost all the parameters used, the aqueous extracts from Mentha piperita showed significantly higher (P < 0.0001) activities than other plants. Antioxidant activity was significantly correlated (P < 0.0001) with TPC among all the plants studied. In addition, the plant extracts showed strong inhibition against pancreatic α-amylase and yeast a-glucosidase. Only Cyperus rotundus and Prosopis farcta have been used traditionally for the treatment of diabetes in Iraq.

Conclusions: In conclusion, to the best of our knowledge, the remaining four plants and herbs (Glycyrrhiza glabra, H. Sabdariffa, Matricaria chamomilla, and M. piperita) have no records in the literature for their antidiabetic effects. Consequently, this study identifies these four medicinal plants as novel inhibitors of key enzymes (α-glucosidase and α-amylase) relevant for type 2 diabetes that showed even higher activity than the currently being used for the treatment of diabetes by the Iraqi herbalists.


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INTRODUCTION

Herbal medicine and traditional phytotherapies have long history of use and acceptance against various diseases and disorders such as cancer, diabetes, malaria, flu, intestinal troubles, blood pressure, kidney, and heart diseases [1] and people from different parts of the world, especially the developing countries including Iraq, have been relying upon for generations. It is interesting to see that the global trend nowadays is going back to the natural way of living and the importance of the traditional phytotherapies is recognized, mainly due to their safety and also due to the various side effects of the currently available synthetic medicines [2]. This growing interest in complementary and alternative medicines is evidenced by the high popularity of the herbal medicine and the herbal shops and health food stores [3]. It is well known that free radicals are the aetiology of a wide range of syndromes and diseases such as liver cirrhosis, atherosclerosis, cancer, and diabetes and that free-radical scavenging agents have the capacity to protect the human body against the damage by the free radicals via modulating the negative impact of these diseases [4]. Oxidative stress, which has been observed in diabetic patients, coexists with a reduction in the antioxidant status, which can enhance the damaging effects of free radicals to the body cells and tissues [5]. Diabetes mellitus is the most serious chronic metabolic disease, constitute a medical problem worldwide and its prevalence has risen at an alarming rate. The most common type of diabetes mellitus is type 2, which accounts for about 95% of all cases [6]. More than 170 million people worldwide suffering from diabetes and this is expected to be doubled by year 2030 [7]. Many complications such as atherosclerosis, myocardial infarction, neuropathy, and nephropathy are associated with diabetes mellitus and these complications appear to be related to the degree of hyperglycemia and subsequent oxidative stress [8]. Moreover, the ability of most of the synthetic compounds used to induce diabetes, such as alloxan, is via inducing oxygen free radicals which damage the pancreas [9]. On the other hand, antioxidants have been shown to reduce the oxidative stress in experimental diabetes and consequently, may be able to reduce the complications associated with diabetes [10].

While the currently available remedies have a wide range of side effects, the need for alternative effective and safe ther-
apeutic strategies without or with minimal side effects is urgent. Medicinal plants and/or their phytochemicals have been used for a long time in controlling postprandial hyperglycemia [3, 11-13]. It is well known that postprandial hyperglycemia is one of the common detected symptoms in type 2 diabetes [14], which occurs when the pancreas fails to produce a sufficient amount of insulin [15]. It is well known that starch is the primary source of blood glucose and postprandial hyperglycemia is related to the amount of consumed starch and its digestion. Consequently, postprandial hyperglycemia can be treated by reducing or slowing dietary carbohydrate digestion and absorption [16-18] via inhibiting starch hydrolizing enzymes [19, 20]. α-amylase is responsible for the digestion of starch in the small intestine and convert them into oligosaccharides which are further hydrolyzed into glucose by another enzyme called α-glucosidases [21]. Consequently, extracts from medicinal plants and/or their derivatives that have the capacity to inhibit α-glucosidase enzyme may be good candidates for the management of diabetes. This study is a part of a research programme aiming at finding a suitable approach to control diabetes by using polyphenolic compounds and other phytochemicals present naturally in medicinal plants and herbs that have the potential to reduce postprandial hyperglycemia. The aim of this study was to evaluate antidiabetic potential of water extracts from selected Iraqi medicinal plants and herbs by determining their antioxidant activities and their inhibitory effects against yeast α-glucosidase and pancreatic α-amylase.

METHODS

Chemicals and Reagents
All chemicals and reagents were obtained from Sigma Chemical Inc., Australia.

Medicinal Plants
Six air-dried medicinal plants and herbs were collected by the second author from different parts of the Middle of Iraq. The plants and herbs were identified by the second author. Their stems, leaves, flowers, and fruits are used for traditional medicine. The scientific names and traditional uses are detailed in Table 1.

Sample Collection and Extraction
About 20-40 g of powdered air-dried leaves, flowers or fruits of the selected plants has been brought from Iraq to New Zealand in January 2011 by the second author in sealed, airtight foil bags for analysis. Briefly, 5 g of the ground sample was mixed with 50 mL of boiling water and then left for 12 h at room temperature with continuous shaking. The sample suspensions were centrifuged (10000 g for 15 min at 10°C) and the supernatants were used for the assays. The filtrates stored at -20°C until analysis.

Antioxidant Activity Assays

Ferric Reducing/Antioxidant Power (FRAP) Assay
The antioxidant activity of water extracts was determined using FRAP assay of Benzie and Strain [22] as a measure of 'antioxidant power' with some modifications [23]. FRAP is a simple test of antioxidant capacity and depends upon reduction of the colorless ferric tripyridyltriazine (Fe³⁺-TPTZ) complex to the ferrous tripyridyltriazine (Fe²⁺-TPTZ) by a reductant at low pH. Fe²⁺-TPTZ has an intensive blue colour and can be monitored at 593 nm.

Aliquots of aqueous plant extracts (8.5 µL) were allowed to react with 275 µL of diluted FRAP reagents using a 96-well microplate and absorbance was read at 593 nm after 30 min incubation at 37 ºC. Only freshly prepared working FRAP reagent were used by mixing 10 volumes of 300 mM/L acetate buffer, pH = 3.6, with 1 volume of 10 mM/L TPTZ (2, 4, 6-triipyridyl-S-triazine) in 40 mM/L HCl and with 1 volume of 20 mM/L ferric chloride, pre-warmed to 37ºC. Standard curve was prepared using different concentrations (500-8000 µmol/L) of FeSO₄ 7H₂O. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentration of Fe²⁺ and were expressed as µmol of FeSO₄ equivalents per g dry weight.

Oxygen Radical Absorbance Capacity (ORAC) Assay
The ORAC assay depends on the free radical damage to a fluorescent probe through the change in its fluorescence intensity, an indication of the extent of damage from its reaction with the peroxyl radical [24]. 2, 2’-Azobis (2-aminopropane) dihydrochloride (AAPH) is used as free radical generator to reduce the fluorescence characteristics of fluorescein, which is used as the fluorescence probe. The reduction in fluorescence is an index of the degree of free radical damage. In the presence of an antioxidant, there is decrease in the change of fluorescence induced by AAPH.

**Table 1**: Medicinal Plants Included in the Study and Their Usage in Traditional Phytotherapy

<table>
<thead>
<tr>
<th>Row</th>
<th>Latin Binomial (Family)</th>
<th>English Name</th>
<th>Local Name</th>
<th>Part Used</th>
<th>Local Medical Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyperus rotundus L. (Cyperaceae)</td>
<td>Nut grass</td>
<td>Alseid</td>
<td>Tuberous roots</td>
<td>Digestive system disorders, diabetes</td>
</tr>
<tr>
<td>2</td>
<td>Glycyrrhiza glabra L. (Fabaceae)</td>
<td>Licorice root/ sweetwood</td>
<td>Aqiq al-sus</td>
<td>Roots</td>
<td>Fever, ulcer, kidney diseases and asthma</td>
</tr>
<tr>
<td>3</td>
<td>Hibiscus sabdariffa L. (Malvaceae)</td>
<td>Roselle</td>
<td>Kuchurat, karkade flowers</td>
<td>Flowers</td>
<td>Hypertension, kidney diseases</td>
</tr>
<tr>
<td>4</td>
<td>Matricaria chamomilla (Asteraceae)</td>
<td>Chamomile</td>
<td>Babooneh</td>
<td>Flowers</td>
<td>Cold and cough, calming agent</td>
</tr>
<tr>
<td>5</td>
<td>Mentha piperita (Lamiaceae)</td>
<td>Peppermint</td>
<td>Nenah</td>
<td>Leaves</td>
<td>Cough, abdominal distension and muscle spasm</td>
</tr>
<tr>
<td>6</td>
<td>Prosopis farcta (Fabaceae)</td>
<td>Dwarf mesquite</td>
<td>Kharnoob</td>
<td>Dry fruits</td>
<td>Diabetes, dysentery, haemorrhoids</td>
</tr>
</tbody>
</table>
The oxygen radical absorbance capacity (ORAC) assay was performed essentially as described by Huang et al. [25] with some modifications. It was carried out in a 96-well plastic plate. Briefly, to all experimental wells, 150 µL of working sodium fluorescein solution was added. In addition, blank wells received 25 µL of 75 mM phosphate buffer, while standards received 25 µL of Trolox dilutions and experimental wells received 25 µL of plant extracts. Then the plate was pre-incubated for 15 min at 37°C. After that, 25 µL of the AAPH solution was added to each well to start the reaction and the plate was put into a plate reader (Victor3TM 1420 Multilabel Counter by Perkin-Elmer Life and Analytical Sciences, Wallac OY, Turku, Finland) to record the signal every 3 minutes for 270 minutes and the microplate was automatically shaken prior to each reading. Excitation and emission wavelengths were set up at 485 and 535 nm, respectively. A total of 90 readings were made during the assay, describing the fluorescein decay. The area under the curve (AUC) was calculated by using the following equation [26]:

$$\text{Where } f_0 \text{ is the initial signal observed (baseline); } f_1 \text{ is the } 1\text{st signal observed and so on. The net AUC was obtained by subtracting the AUC of the blank from that of the standard or the experimental incubations. A blank using phosphate buffer instead of the antioxidant and calibration solutions of Trolox (12.5, 20, 50, and 100 µM) as antioxidant were carried out in each assay. The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net AUC. ORAC values were expressed as mg Trolox equivalent (TE) per g of dry weight (DW) of the plant. If the fluorescence of the final reading has not declined by more than 95% from the first reading, the diluted sample is reanalyzed until a satisfactory fluorescent reading is achieved [26]. Accordingly, a series of dilutions were made for this purpose.}

**Total Polyphenols Contents Estimation by the Folin-Ciocalteau Method**

The total phenolic content (TPC) of each plant sample was quantified according to the method of Molan et al. [23]. Aliquots of extracts (12.5 µL) were mixed with 250 µL of 2% Na2CO3 solution in 96-well microplates and allowed to sit at room temperature for 5 minutes. After that, 12.5 µL of 50% aqueous Folin-Ciocalteau’s phenol reagent was added, and the plate was allowed to sit at room temperature for a further 30 minutes at room temperature prior to reading the absorbance at 650 nm using a plate reader. Polyphenol content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of dry weight.

**α-Glucosidase Inhibition Assay**

The assay was conducted as described previously [27, 28] with some modifications. This assay uses p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate, which is hydrolyzed by α-glucosidase to release p-nitrophenol. In this assay, 20 µL of a sample solution was mixed with 70 µL of the enzyme solution (0.6 units/mL) in 0.1 M phosphate buffer (pH = 6.8) and incubated at 37°C for 10 minutes. After incubation, 100 µL of 4 mM pNPG solution in the above buffer was added to initiate the colorimetric reaction at 37°C. Enzymatic activity was monitored by measuring the absorbance at 405 nm in a microplate reader (ELx808 BioTek Instruments Inc, USA). The released p-nitrophenol was monitored at zero time and then after incubation at 37°C for 30 minutes. Acarbose was used as a positive control and water as a negative control. Experiments were conducted in triplicates. The absorbance at zero time was subtracted from the absorbance value at 30 minutes in order to get the actual absorbance and then the percentage of enzyme inhibition by the plant extracts was calculated by the following equation:

**Amylase Inhibition Activity**

The α-amylase inhibitory activity was determined according to the method described by Gowri et al. [29]. With some modifications. 200 µL of aqueous extracts were pre-incubated with 200 µL of porcine pancreatic α-amylase (4.0 Unit/mL; prepared in ice cold water) for 5 min and then 400 µL of 0.5% (w/v) soluble starch solution was added. The mixture was incubated at 25°C for 3 min and the reaction was terminated by adding 400 µL of dinitrosalicylic acid colour reagent (1.0 g of 3, 5-dinitrosalicylic acid, 30 g of sodium potassium tartrate and 20 mL of 2N NaOH to a final volume of 100 ml Milli-Q water). The mixtures were then reincubated in a boiling water bath (85-90°C) for 10 min in order to develop colour. After cooling to room temperature, 50 µL of reaction mixture was then diluted by adding 175 µL of Milli-Q water, and absorbance was measured at 550 nm with a microplate reader (ELx808 BioTek Instruments Inc, USA). The reading was compared to the control, which contained 200 µL of water instead of plant extract. Individual blanks were used to correct for the background absorbance due to test samples. The α-amylase inhibitory activity was expressed as percentage inhibition of enzyme activity and was calculated using the following equation:

**Statistical Analysis**

Results were expressed as mean ± standard error. The mean of triplicate determinations of two separate experiments and standard errors are presented. One-way analysis of variance (ANOVA) and Tukey’s test were used for mean comparisons and P < 0.05 was considered to be statistically significant. Simple linear regression analysis (with coefficients of determination, R²) was done to determine the correlation between two variables. All statistical tests were analyzed using the SAS program for Windows version 9.2 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**Description of the Medicinal Plants Used in This Study**

Table 1 contains the information of the plants used in the present study. In Iraqi traditional medicine, when these herbs are used for patients, recommended parts are mixed with boiled water and infusions prepared are given to the patients orally.

**Total Phenolic Content (TPC) and Total Flavonoids Content (FTC)**

The total phenolic content (TPC) in the extracts of the six medicinal plants was determined from a linear Gallic acid standard curve and expressed as mg of gallic acid equivalent.
The total phenolic content (mg of Gallic acid equivalent g⁻¹ of dry weight) of the tested plants (Table 2). The TPC values of the 6 plants varied from 15.7 to 85.1 mg GAE g⁻¹ DW. The aqueous extracts from Mentha piperita showed the highest TPC (85.03 mg GAE g⁻¹ DW) (P < 0.0001) while the extract from the tuberous roots of Cyperus rotundus showed the lowest TPC (15.7 mg GAE g⁻¹ DW).

### Antioxidant Activity as Assessed by Ferric Reducing Antioxidant Power (FRAP) Assay

Antioxidant activity as measured by FRAP assay showed a wide range of variation among the plants studied (122.5-618.5 μmol of FeSO₄ equivalent g⁻¹ DW, Table 3). As in FRAP assay, the extracts from M. piperita showed the highest (P < 0.0001) activity (201.6 mg trolox equivalent g⁻¹ DW). The TPC values of the 6 plants varied from 15.7 to 85.1 mg TE g⁻¹ DW), followed by Hibiscus sabdariffa (82.5 μmol of TE g⁻¹ DW), Prosopis farcta (48.2 mg TE g⁻¹ DW) and Cyperus rotundus (39.8 mg TE g⁻¹ DW). There was a significant correlation (R² = 0.9735, P < 0.0001) between the TPC and the ORAC values (Table 4). In addition, there was a significant positive linear correlation (Table 4) between the total phenolic content and the ferric reducing antioxidant power (FRAP) (R² = 0.9482, P < 0.0001).

### Antioxidant activity as assessed by Oxygen Radical Absorbance Capacity (ORAC) Assay

The antioxidant activities as measured by ORAC are shown in Table 3. As in FRAP assay, the extracts from M. piperita showed the highest (P < 0.0001) activity (201.6 mg trolox equivalent (TE) g⁻¹ DW), followed by Hibiscus sabdariffa (82.5 mg TE g⁻¹ DW), Mentha piperita (39.8 mg TE g⁻¹ DW) and Cyperus rotundus (39.8 mg TE g⁻¹ DW). There was a significant correlation (R² = 0.9735, P < 0.0001) between the total phenolic content and the ORAC values (Table 4). In addition, there was a significant positive linear correlation (Table 4) between the ORAC values and the ferric reducing antioxidant power (FRAP) (R² = 0.9482, P < 0.0001).

### Table 2: Total Phenolic Contents of Aqueous Extracts of 6 Medicinal Plants Grown in the Middle of Iraq

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Total Phenolic Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyperus rotundus L.</td>
<td>15.7 ± 4.7</td>
</tr>
<tr>
<td>Glycyrrhiza glabra L.</td>
<td>30.5 ± 1.6</td>
</tr>
<tr>
<td>Hibiscus sabdariffa L.</td>
<td>45.9 ± 0.2</td>
</tr>
<tr>
<td>Matricaria chamomilla</td>
<td>50.9 ± 1.2</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>85.1 ± 3.4</td>
</tr>
<tr>
<td>Prosopis farcta</td>
<td>17.3 ± 1.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE of n = 6 replicates per plant extract. Total phenolic content (mg of Gallic acid equivalent g⁻¹ of dry weight).

### Table 3: Antioxidant Activity of Aqueous Extracts of 6 Medicinal Plants Grown in the Middle of Iraq

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Antioxidant Activity as Measured by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRAP (µmol of FeSO₄ equivalent g⁻¹ dry weight)</td>
</tr>
<tr>
<td>Cyperus rotundus L.</td>
<td>122.5 ± 3.2</td>
</tr>
<tr>
<td>Glycyrrhiza glabra L.</td>
<td>200 ± 1.8</td>
</tr>
<tr>
<td>Hibiscus sabdariffa L.</td>
<td>232 ± 6.4</td>
</tr>
<tr>
<td>Matricaria chamomilla</td>
<td>391.1 ± 11.2</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>618.5 ± 1.6</td>
</tr>
<tr>
<td>Prosopis farcta</td>
<td>205 ± 9.1</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE of n = 6 replicates per plant extract. FRAP (µmol of FeSO₄ equivalent g⁻¹ dry weight). ORAC (mg of Trolox equivalent/g dry weight).

### Table 4: Correlation Between Total Phenolic Content and the Antioxidant Activity Determined by FRAP and ORAC Methods

<table>
<thead>
<tr>
<th>Antioxidant Activity</th>
<th>Coefficient of Determination (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP</td>
<td>0.9677</td>
</tr>
<tr>
<td>ORAC</td>
<td>0.9735</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>0.6158</td>
</tr>
<tr>
<td>α-amylase</td>
<td>0.1971</td>
</tr>
</tbody>
</table>

### Table 5: Inhibitory Activity Against α-Amylase and α-Glucosidase

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>% Inhibition Against α-Amylase</th>
<th>% Inhibition Against α-Glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyperus rotundus L.</td>
<td>12.8 ± 1.2</td>
<td>33.6 ± 1.9</td>
</tr>
<tr>
<td>Glycyrrhiza glabra L.</td>
<td>24.0 ± 2.1</td>
<td>59.5 ± 1.4</td>
</tr>
<tr>
<td>Hibiscus sabdariffa L.</td>
<td>98.5 ± 1.5</td>
<td>61.3 ± 2.4</td>
</tr>
<tr>
<td>Matricaria chamomilla</td>
<td>28.4 ± 3.3</td>
<td>44.5 ± 2.2</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>58.7 ± 1.2</td>
<td>84.7 ± 0.8</td>
</tr>
<tr>
<td>Prosopis farcta</td>
<td>43.0 ± 3.2</td>
<td>41.1 ± 1.35</td>
</tr>
</tbody>
</table>

% inhibition at 5 mg/ml of assays reagent.

Water extracts from the leaves of Mentha piperita showed the highest FRAP activity (P < 0.0001) while the aqueous extracts from the dry roots of Cyperus rotundus showed the lowest FRAP value (Table 3). A significant positive correlation (R² = 0.9735, P < 0.0001) was found between TPC and the ferric reducing antioxidant power (FRAP) (Table 4).

### Inhibition of α-Glucosidase and α-Amylase Enzymes

Evaluating the inhibitory potential of crude extracts from the tested medicinal plants and herbs against α-glucosidase showed that the crude water-soluble extracts from M. piperita exhibited the highest inhibitory (P < 0.0001) activity (84.7%) followed by Hibiscus sabdariffa (61.3%), Glycyrrhiza glabra (59.5%), Cyperus rotundus (44.5%), Prosopis farcta (41.1%) and Matricaria chamomilla (33.6%) (Table 5). Table 5 also shows that the aqueous extracts from the tested plants and herbs were able to inhibit the activity of α-amylase by 12.8-98.5% at 5 mg/mL. The crude extracts from H. Sabdariffa showed the highest (P < 0.0001) inhibitory activity (98.5%) followed by M. piperita (58.7%), P. farcta (43%), G. glabra (24%), M. chamomilla (18.4%) and C. rotundus (12.8%).

Our results showed that the inhibitory activity of the tested plants against α-glucosidase was positively correlated (R² = 0.6158) with the total phenolic contents (TPC). Although the correlation between the TPC and the inhibitory activity...
against α-amylase was positive ($R^2 = 0.1971$), it was weaker than that between TPC and α-glucosidase (Table 4).

### DISCUSSION

In this study, we investigated six medicinal plants and herbs, commonly used in the traditional medicine in Iraq, for antioxidant activity, phenolic contents, and the possible inhibitory activity against α-glucosidase and α-amylase enzymes as a part of our continuing study of medicinal plants that may have antidiabetic effect, and other biological activities in order to provide the scientific base of their application and to pave the way for clinical research work of the indigenous plants to prove and authenticate the traditional phytotherapies.

Among the six tested medicinal plants, Mentha piperita showed the highest TPC and highest antioxidant activities as measured by FRAP and ORAC assays. At 5 mg/mL crude water-soluble extracts from this herb showed the highest inhibitory activity against α-glucosidase while the inhibitory activity against pancreatic a-amylase was found to be second after roselle (Hibiscus sabdariffa). Zheng and Wang [30] screened 27 culinary herbs and 12 medicinal herbs including M. piperita for their antioxidant activity and total phenolic contents and reported that the ORAC value for an aqueous solution of previously frozen fresh M. piperita leaves was among the highest found in an analysis of popular medicinal herbs grown in Washington, DC, USA. M. piperita is a perennial herb which has a worldwide distribution and one of the most widely consumed single ingredient herbal teas. The phenolic constituents of the leaves include rosmarinic acid and several flavonoids, primarily eriocitrin, luteolin and hesperidin and under in vitro conditions, peppermint has significant antimicrobial and antiviral activities, strong antioxidant and antitumor actions [31].

In a study using the ferric reducing antioxidant power (FRAP) assay, Dragland et al. [32] found the relative antioxidant value of dried M. piperita (78.5 mmol of FeSO4 equivalent/100 g) to be the third after oregano (Origanum vulgare) and sage (Salvia officinalis). Using the same assay, our results showed that water extracts from the dried leaves of M. piperita showed the highest FRAP values among the tested medicinal plants. Based on mmol/100 g weight, our FRAP value was 61.9 mmol/100 g dry weight which is lower than that reported by Dragland et al. [32] for the same herb grown in Norway. This may be explained by the differences in environmental conditions between the two countries.

Licorice root from Glycyrrhiza glabra is a traditional medicine and has a long history of use and acceptance and since more than 2000 years the preparation and application of aqueous licorice extracts is well documented and has been described as ‘the grandfather of herbs’ [33]. Although licorice root is used mainly for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases, several other useful pharmacological properties such as anti-inflammatory, antiviral, antimicrobial, antioxidative, anticaner activities, hepatoprotective and cardioprotective effects have also been documented [34].

In addition to the high antioxidant activity, the present study showed that aqueous licorice extracts have potent inhibitory activity against α-glucosidase (59.5%) and α-amylase (24%) enzymes. This novel finding adds another important pharmacological property for licorice as a novel anti-diabetic agent. Various components have been isolated from licorice such as triterpene saponins, flavonoids, isoflavonoids, with glycyrrhizinic acid normally being considered to be the main biologically active component [34]. Haraguchi et al. [35], reported that the isoflavone derivatives of G. glabra such as glabridin inhibited lipid peroxidation in rat liver microsomes and protected mitochondrial functions from oxidative stresses. It has been suggested that most of the biological activities of licorice roots are attributed to the potent antioxidant activity and to the capacity of certain licorice constituents, such as glycyrrhizin and glabridin to inhibit the generation of reactive oxygen species [36].

Cyperus rotundus is widely distributed in the Mediterranean basin areas. This plant is a traditional herbal medicine used widely by Indians, Chinese and Japanese as analgesic, sedative, antispasmodic, antimalarial, stomach disorders and to relieve diarrhoea [37, 38]. In Iraq, the dried rhizomes of this plant are used for treating fever, digestive system disorders (nausea, diarrhea, and others) [39]. The rhizomes contain Flavonoids, alkaloids, saponins and fatty oils (glycerides) [39]. In addition to the high antioxidant activity, the present study showed that water-soluble crude extracts prepared from the dried rhizomes of this plant were able to inhibit the activity of α-glucosidase by 33.6% and that of α-amylase by 12.8%. Recently, Kilani-Jaziri et al. [40] investigated the antioxidant, antimicrobial and antigenotoxic activities of extracts from the aerial parts of C. rotundus and reported that the extracts possess potent antimicrobial, antioxidant and antigenotoxic activities, which could be derived from compounds such as flavonoids and phenols. The inhibitory activity against α-glucosidase and α-amylase may provide a scientific evidence for the use of this medicinal plant against diabetes by the Iraqi herbalists.

There is a long history of traditionally using the extraction of Prosopis farcta plant for treatment of angina pectoris in Iran [41]. Our results showed that crude water extracts prepared from the dried fruits have the ability to scavenge the oxygen radical species (ORS) as evidenced by the high ORAC value (48.2 µmol of Trolox equivalent/g dry weight). The same extracts were able to reduce the activity of α-glucosidase and α-amylase by 41% and 43%, respectively and these results validate the traditional use of this medicinal plant for the treatment of diabetes by the Iraqi herbalists.

From the traditional medicine point of view, the flowers of roselle (H. Sabdariffa) are the most important parts with various medicinal applications have been developed in different parts of the world [42] and they are commonly used to make teas, jellies and jams due to the brilliant red color and unique flavour which make it a valuable food product. Hibiscus tea is a caffeine free herbal tea which is made out of the dried fruit part of roselle which is rich in color and tastes like berries. It has been used to treat hypertension, liver damage, leukemia, cancer, and lower blood pressure [43]. In Iraq, it is called Karkade and sun-dried flowers from H. Sabdariffa are used to prepare a popular tea that is also traditionally used by the population for the treatment of obesity [44]. Many chemical compounds have been found in the flowers such as phenolic compounds, anthocyanins, and hibiscus acid [45].

In addition to the potent antioxidant activity, the present results showed that the water extracts prepared from the flowers of roselle have the highest inhibitory activity against the
a-amylose and a-glucosidase enzymes. Recently, Mohd-Esa et al. [42] studied the DPPH-radical scavenging activity of different parts of this herb and found that the extracts from the calyx were able to scavenge DPPH-radical by 31% which was lower than that for the extracts prepared from the seeds, leaves, and stems. It has been found that 50% aqueous methanolic extract of roselle has high inhibitory activity against pancreatic a-amylace [46]. In addition, some studies showed that hibiscus acid and cyanidin-3-glucoside from roselle are active pancreatic a-amylase inhibitors [47, 48]. Accordingly, it can be concluded that the inhibitory effect of roselle on pancreatic a-amylase and a-glucosidase enzymes reported in the present study and other studies may be related to these phytochemicals. Matricaria chamomilla (Roman chamomile) is a fragrant, perennial herb and is frequently employed as an ingredient in numerous herbal remedies. The flowers of Matricaria chamomilla are used medicinally to treat a variety of disorders from indigestion to insect bites. In the present study, crude water extracts were also able to inhibit the activity of both a-amylase and a-glucosidase enzymes with 33.6% and 18.4%, respectively.

In general, the antioxidant activity (as measured by FRAP and ORAC) was significantly correlated with TPC among all the plants studied. These findings indicate that polyphenols may be important contributors to the antioxidant activities of water extracts. Our results are in line with other studies conducted on other medicinal plants and herbs grown in other countries [49, 50]. It is well known that inhibition of a-glucosidase and a-amylase activities leads to retardation of starch hydrolysis, resulting in delayed rise in postprandial hyperglycemia and therefore can be an important strategy in the management of type 2 diabetes [51]. Our results showed that the inhibitory activity of the tested plants against both a-amylase and pancreatic a-amylase was positively correlated with the total phenolic compounds contents. Similarly, some studies have shown that the inhibitory activities of plant-based extracts or products against a-glucosidase and pancreatic a-amylase can be linked to the total phenolic compounds and flavonoids contents [52, 53]. These correlations may indicate the importance of phenolic compounds as mediators for the inhibitory effects of plant-based foods on a-amylase and a-glucosidase. Some of the tested plants represent a very interesting source of antiradical phytochemicals that may be responsible for the activity again the diseases claimed by the local folk healers who use these plants with a very high confidence which is based on a long accumulative experience. Antioxidant activity (as measured by FRAP and ORAC) was significantly correlated with TPC among all the plants studied indicating that phenolic compounds are the significant contributors to the antioxidant activity of the medicinal plants studied. In addition, the tested plants showed strong inhibitory activities against pancreatic a-amylase and yeast a-glucosidase. Among the tested plants and herbs, only Cypers rotundus and Prosopis farcta have been used traditionally for the treatment of diabetes in Iraq and some countries in the Middle East. To our knowledge, the remaining three plants and herbs (Anthe mis nobilis, Glycyrrhiza glabra and Mentha piperita) have no records in the literature for their antidiabetic effects. Consequently, this study identified these three medicinal plants and herbs as novel inhibitors of key enzymes (a-glucosidase and a-amylose) relevant for type 2 diabetes. We are planning to conduct further research to isolate the antioxidant components of the plants with high activity and test their antioxidant activity in comparison with their parent plants.

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CONFLICTS OF INTEREST
There is no conflict of interests.

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