# Antioxidant activity and α-amylase inhibitory effect of selected medicinal plants grown in jordan: an in-vitro study

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**Background/aim**
Olive tree (*Olea europaea*), *Artemisia herba-alba*, *Rosmarinus officinalis*, and *Teucrium polium* are among the medicinal plants grown in Jordan and used in folk medicine to reduce the complication of diabetes. This study aimed to evaluate the antioxidant activity and inhibitory activity of phenolics and porcine pancreas α-amylase, *in vitro*, of these medicinal plants.
**Materials and methods**
The plant materials of *A. herba-alba*, *O. europaea*, *R. officinalis*, and *T. polium* were purchased from the local market in Amman. The aqueous extracts of aerial parts were investigated for the total phenolics and flavonoidal contents spectrophotometrically. Antioxidant activity was determined by two methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power activity using ferricyanide assays. α-Amylase inhibitory activity was determined using 2-chloro-4-nitrophenyl-α-d-maltotrioside (CNP-$G_3$) assay *in vitro*.
**Results**
Aqueous extract of *R. officinalis* was the highest in phenolic and flavonoidal contents (107.6 mg gallic acid equivalent/100 g and 482 mg quercetin equivalent/100 g, respectively) among other three extracts, and it has the highest reducing power and DPPH inhibition activities at concentration of 25 ppm (30.9 and 57.3%, respectively). Moreover, it showed a remarkable α-amylase inhibitory activity (70% at a concentration of 20 μg/ml). *T. polium* aqueous extract showed to contain the least amounts of phenolics and flavonoidal compounds of 43.4 mg gallic acid equivalent /100 g and 206.0 mg quercetin equivalent/100 g, respectively; the lowest reducing power and DPPH inhibition activities of 14.8 and 38.3%, respectively, at concentration of 25 ppm; and weak α-amylase inhibitory activity (5% at a concentration of 20 μg/ml). However, all extracts including *O. europaea* and *A. herba-alba* showed a potential antioxidant activity better than the standard BHT (butylated hydroxytoluene) on DPPH (2,2-diphenyl-1-picrylhydrazy) radical scavenging assay. Phenolic compounds from the studied plants showed strong and significant correlation with both reducing power activity ($r$=0.92, $P<0.01$) and α-amylase inhibitory activity ($r$=0.98, $P<0.01$) at 180 s.
**Conclusion**
Aqueous extract of *R. officinalis*, olive tree leaves (*O. europaea*), and *A. herba-alba* may be suggested as potential sources of natural antioxidants and α-amylase inhibitory activity owing to their high phenolic and flavonoidal contents.

**Keywords:**
*Artemisia herba-alba*, *Olea europaea*, *Rosmarinus officinalis*, *Teucrium polium*, α-amylase inhibitory activity

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## Introduction

Medicinal plants and herbs are used in many parts of the world to treat various illnesses of humankind such as diabetes, blood infections, skin irritations, gastrointestinal disorders, and others and considered the basis of traditional medicine [1,2]. Free radicals are the main cause of several chronic diseases such as heart disease, stroke, and cancer, and the antioxidants isolated from different medicinal plants have been shown to scavenge free radicals and have potential therapeutic effect on free radical-associated disorders [3]. In recent years, increasing awareness to investigate naturally occurring antioxidants and antimicrobials results from the increasing demand of consumer for food products free from synthetic chemical additives. In Jordan, more than 2500 plant species with ~500 genera belonging to 100 families are classified as medicinal plants; among these, 70 plant species are used by local diabetic patients [4]. Jordan has the ninth

highest incidence of diabetes among neighboring countries, with a prevalence rate of 10.1% [5].

Diabetes type 2 is one of the primary risks that threaten human health owing to its chronic complications. Pancreatic α-amylase is an enzyme of the digestive system that catalyzes the hydrolysis of starch to produce glucose, maltose, oligosaccharides, and dextrins, and leads to elevated postprandial glucose levels [6]. The inhibition or the delay of such enzymes secretion can play a role in the control of diabetes. Synthetic hypoglycemic agents can cause serious adverse effects, and in many cases, they fail to elevate diabetic complications [7]. Herbal remedies all over the world are getting more recognition for the treatment of several diseases including diabetes as they are cheaper and have low adverse effects when compared with synthetic chemical drugs [8,9].

*Artemisia herba-alba* is a greenish-silver perennial herb that belongs to the family Asteraceae. It was known in folk medicine for its medicinal properties for treatment of colds, cough, intestinal disturbances, antidiabetic agent diarrhea, bronchitis, neuralgias, and hypertension [10,11].

The leaves of *Olea europaea* have been widely used in traditional remedies in European and Mediterranean countries as an extract and herbal teas, owing to their antioxidant, antihypertensive, anti-inflammatory, antiatherogenic, hypoglycemic, and hypocholesterolemic properties [12]. The main phenolic compound in *O. europaea* leaves is oleuropein, which is responsible for the bitter taste of the fruits and the leaves of olive plant, and several studies have reported that oleuropein has an antihyperglycemic effect in diabetic rats [13,14].

Rosemary (*Rosmarinus officinalis L.*) belongs to the family Lamiaceae and is a famous aromatic plant used for different medicinal purposes. Rosemary extracts have a variety of pharmacological activities, such as antioxidant, antimicrobial, antidiabetic, cognition improving, cancer chemopreventive, DNA protective, choleretic, hepatoprotective, and a stimulant and mild analgesic, and it has been considered effective for treating headaches, poor circulation, inflammatory diseases, and physical and mental fatigue [15]. Most of these effects of rosemary are the consequence of high antioxidant activity of its chemical constituents, which include carnosol, carnosic acid, rosmarinic acid, ursolic acid, and caffeic acid. The strong antioxidant properties of rosemary have been mainly referred to its major diterpenes, carnosol and carnosic acid, and to the essential oil components. Rosemary is one of the dietary components that is known as safe and used in our food products [16].

*Teucrium polium* (Jordanian Ja'adeh) is one of more than 300 species of the genus *Teucrium* from Lamiaceae family [17]. *T. polium* is used in traditional medicine for different types of pathological conditions, like gastrointestinal disorders, inflammations, rheumatism, and diabetes [18]. The therapeutic benefits are usually referred to the ability of *T. polium* extracts to suppress oxidative processes, and various investigators have shown that *T. polium* extract reduces blood glucose by mechanisms such as enhancement of peripheral metabolism of glucose rather than increase in insulin release [19].

This study aimed to evaluate the antioxidant activity and α-amylase inhibitory activity, *in vitro*, of selected four medicinal plants grown in Jordan, which are used in folk medicine to reduce the complication of diabetes: *A. herba-alba*, *O. europaea*, *R. officinalis*, and *T. polium.*

## Materials and methods

### Plant material

The plant material of the present study, *A. herba-alba*, *O. europaea*, *R. officinalis*, and *T. polium*, were purchased from local herbal shops in Amman. The botanical identification was done by Dr Khalid Abu-Laila (botanist). The English, Arabic, and Latin names of plant material of the present study are listed in Table 1.

### Preparation of plant aqueous extracts

The plant extracts were prepared by soaking 10 g of the dried plant material with 100 ml preboiled distilled water and keeping the extract for 24 h at room temperature. After filtration through the filter paper, the volume of the filtrate was increased to 100 ml with distilled water to obtain 10% aqueous stock solution.

### Determination of yield content in plant extracts

The yield content (%) of aqueous plants extracts was determined in duplicates using a conventional oven at 105°C until constant weight was achieved.

**Table 1 English, Arabic, and Latin names of plants used in the study**

| English names | Arabic names | Latin names |
|---|---|---|
| White wormwood | Shieh | *Artemisia herba-alba* |
| Olive leaves | Zaitoon | *Olea europaea* |
| Rosemary | Hassa alban | *Rosmarinus officinalis* |
| Mountain germander | Ja'adeh | *Teucrium polium* |

#### Determination of total phenolic content

The total phenolic content present in water extracts of the selected plants was determined using Folin–Ciocalteau reagent [20]. Briefly, 0.1 ml of each plant extract (10 g/100 ml) was transferred into a 10 ml volumetric flask, followed by addition of 2.5 ml of distilled water, and then, 250 µl Folin–Ciocalteu reagent was added, followed by mixing thoroughly for 3 min. After that, a 0.5 ml of 10% sodium carbonate (10 g/100 ml) was added and the volume was completed to 10 ml with distilled water. The absorbance was measured at 760 nm spectrophotometrically (Model UVD-2950; Labomed Inc., Los Angeles, CA, USA). Gallic acid was used as the standard for a calibration curve. The total phenolic compound contents (mg/100 g) were expressed as gallic acid equivalent (GAE) and determined from the following regression equation based on the established calibration curve:

$$Y=0.079x-0.019,\ R^2=0.995,$$

where $Y$ is the absorbance, and $x$ is the gallic acid concentration in ppm. All measurements were done in duplicates.

#### Determination of flavonoidal content

The content of flavonoids was determined by the Miliuskas method [21]. Briefly, 1.0 ml of each plant extract (10 g/100 ml) was mixed with 1.0 ml of 2% aluminum chloride in water solution (20 g/l) and diluted with distilled water to 25 ml. The absorption at 415 nm was taken after 40 min at 20°C (Model UVD-2950). The amount of flavonoids in water extracts of different plants was expressed as quercetin equivalent (QE) and determined from the following regression equation based on the established calibration curve:

$$Y=0.093x-0.039,\ R^2=0.987,$$

where $Y$ is the absorbance, and $x$ is the quercetin concentration in ppm. All measurements were done in duplicates.

#### Determination of antioxidant activities

##### *1,1-Diphenyl-2-picrylhydrazyl free radical scavenging assay*

DPPH (1,1-diphenyl-2-picrylhydrazyl) was used to determine the free radical scavenging activity in the water extracts of the plants using the method of Hatano [22]. Overall, 10, 25, 50, 100, 150, and 200 µl of each plant extract (10 g/100 ml) was completed with methanol to reach 2 ml, then mixed with 2 ml of methanolic solution of DPPH ($6\times10^{-5}$ mol/l), and the mixture was mixed by Vortex. The absorbance of each extract, standard (200 ppm BHT), and control was measured at 517 nm (Model UVD-2900) after 30 min against blank that was prepared from similar concentrations for each treatment to eliminate the effect of the samples color on the optical density:

$$\text{DPPH scavenging activity}\,(\%) = \frac{\text{Absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\times 100.$$

##### *Reducing power activity*

The reducing power of the plant water extracts was determined using the Yildirm method [23]. Different amount of each extract (equivalent to 30 µg of ascorbic acid) was mixed with 2.5 ml phosphate buffer (0.2 mol/l, pH 6.6) and 2.5 ml of potassium ferricyanide (1 g/100 ml). The mixture was then incubated at 50°C for 30 min followed by addition of 2.5 ml trichloroacetic acid (10 g/100 ml), and the mixture was then centrifuged at 1650*g* for 10 min. After that, 2.5 ml of the upper layer solution was taken and mixed with 2.5 ml of ferric chloride (0.1 g/100 ml). The absorbance was measured at 700 nm for the water extracts of each of the selected plants and standard of ascorbic acid (30 µg):

$$\text{Antioxidant activity}(\%) = \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{ascorbic acid}}}\times 100.$$

##### *Determination of α-amylase inhibitory activity by 2-chloro-4-nitrophenol-G3 assay*

The ability of porcine pancreas α-amylase to release 2-chloro-4-nitrophenol (CNP) from CNP-$G_3$ was measured according to Suganuma *et al.* [24]. A reaction mixture (450 µl) consisting of 0.15 mmol/l CNP-$G_3$ and 0.2 mol/l potassium thiocyanate solution in 0.05 mol/l phosphate buffer, pH 7.0, and 3.3 mg/ml (30 µl) aqueous solution of the extract was preincubated for 5 min at 25°C, followed by the addition of 20 µl of freshly prepared α-amylase solution (1 mg/ml) in phosphate buffer, pH 7.0. The liberation of CNP from CNP-$G_3$ was determined by measuring an increase of the absorbance at 405 nm during the reaction.

$$\text{Inhibitory activity}\,(\%)=[(A-B)/A]\times 100,$$

where $A$ is the increase in the absorbance during the reaction in the absence of the extract and $B$ is in its presence.

#### Statistical analysis

Statistical calculations were performed using statistical analysis system, statistical package for the social

sciences program, version 20 (SPSS Inc., Chicago, Illinois, USA). Significant differences among mean of treatments were determined using LSD test. Differences at *P* less than 0.05 were considered significant. All treatments were conducted in duplicates.

## Results

### Determination of yield content in plant extracts

The data obtained in Table 2 show the yield content of plant extracts in water as solvent. The extract of *A. herba-alba* gave the highest yield (13.783 ±0.210 g/100 g DW) followed by *R. officinalis* (11.801±0.106 g/100 g DW) and *O. europaea* (9.748±0.002 g/100 g DW), whereas the *T. polium* extract gave the least yield content (8.56± 0.10 g/100 g DW). This disparity in the result is owing to the phytochemical constituents of the plants. The extraction method with proper solvent is considered as an important step for bioactive components extraction from any plant material [25].

### Total phenolic content

The average contents of phenolic compounds of aqueous plants extracts as GAE are presented in Table 3. The results show that *R. officinalis* extract has significantly the highest phenolic content value of 107.63±1.30 mg GAE/100 g DW, followed by *O. europaea* and *A. herba-alba* extract with value of 90.402±0.597 and 59.415±0.261 mg GAE/100 g DW, respectively. *T. polium* extract contained the lowest phenolic content value of 43.39±0.73 mg GAE/100 g DW.

**Table 2 Average yield content (g/100 g DW) of aqueous plant extracts**

| Extracts | Yield content |
|---|---|
| *Artemisia herba-alba* | 13.783±0.210 |
| *Olea europaea* | 9.748±0.002 |
| *Rosmarinus officinalis* | 11.801±0.106 |
| *Teucrium polium* | 8.56±0.10 |

All data are presented as mean±SD of duplicate analysis. $P<0.05$, mean in the column followed by different letters are significantly different.

**Table 3 Average total phenolic content (mg GAE/100 g DW) of aqueous plant extracts**

| Extracts | Phenolic content |
|---|---|
| *Artemisia herba-alba* | 59.415±0.261[c] |
| *Olea europaea* | 90.402±0.597[b] |
| *Rosmarinus officinalis* | 107.63±1.30[a] |
| *Teucrium polium* | 43.39±0.73[d] |

All data are presented as mean±SD. $P<0.05$, values having different letters are significantly different.

### Total flavonoids content

The average contents of flavonoids of the aqueous plants extracts as QE are presented in Table 4. The highest significant flavonoid contents were found in *R. officinalis* at 481.99±10.30 mg QE/100 g DW, followed by *A. herba-alba* at 402.52±1.13 mg QE/ 100 g DW, *O. europaea* at 344.68±3.91 mg QE/ 100 g DW, and *T. polium* extract at 205.95± 1.81 mg QE/100 g DW, which has the lowest flavonoid contents.

### 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity

Figure 1 shows the effect of different concentrations from aqueous plants extracts on DPPH radical scavenging activity expressed as a percentage of inhibition of the DPPH free radical. The results described that the scavenging effects of all plants extracts on DPPH radical increased in concentration-dependent manner.

### Reducing power activity

Figure 2 shows the reducing power for the aqueous plants extracts expressed as 30 μg vitamin C equivalent. The weakest reducing power activity was exhibited by *T. polium* extract (14.76%). Reducing power activity of *R. officinalis* extract was superior to all extracts tested with a percentage of inhibition value of 30.89%. On the contrary, none of the extracts showed activity as strong as the standard ascorbic acid (100%).

### α-Amylase inhibitory activity

Table 5 shows the inhibitory activity of plants extracts on porcine pancreas α-amylase after 2 min. *R. officinalis* has significantly higher inhibitory effect on α-amylase

**Table 4 Average of total flavonoids content (mg quercetin equivalent/100 g DW) of aqueous plant extracts**

| Extracts | Flavonoids content |
|---|---|
| *Artemisia herba-alba* | 402.52±1.13[b] |
| *Olea europaea* | 344.68±3.91[c] |
| *Rosmarinus officinalis* | 481.99±10.30[a] |
| *Teucrium polium* | 205.95±1.81[d] |

All data are presented as mean±SD. $P<0.05$, values having different letters are significantly different.

**Table 5 α-Amylase inhibitory activity (%) of aqueous plant extracts at 20 ppm concentration after 2 min**

| Plant extracts | Inhibition (%) |
|---|---|
| *Artemisia herba-alba* | 26.34±4.53[c] |
| *Olea europaea* | 45.59±1.58[b] |
| *Rosmarinus officinalis* | 76.565±1.26[a] |
| *Teucrium polium* | 5.815±4.22[d] |

All data are presented as mean±SD of duplicate analysis. $P<0.05$, mean in the column followed by different letters are significantly different.

with inhibition of ~76% after 2 min at concentration of 20 ppm, followed by inhibitory activity of ~45 and 26% from *O. europaea* and *A. herba-alba*, respectively, whereas *T. polium* showed the lowest inhibitory activity at 5.8%.

### Correlation between total phenolic and flavonoidal contents and 1,1-diphenyl-2-picrylhydrazyl, reducing power, and α-amylase activities

The data presented in Table 6 shows that phenolic compounds at 100 ppm significantly correlated with both reducing power activity ($r$=0.927, $P$<0.01) and α-amylase activity at 180 s ($r$=0.98, $P$<0.01). On the contrary, flavonoid compounds at 1000 ppm were found to have significant correlation with DPPH activity at 50 ppm ($r$=0.95, $P$<0.01) and α-amylase activity at 180 s ($r$=0.84, $P$<0.01).

## Discussion

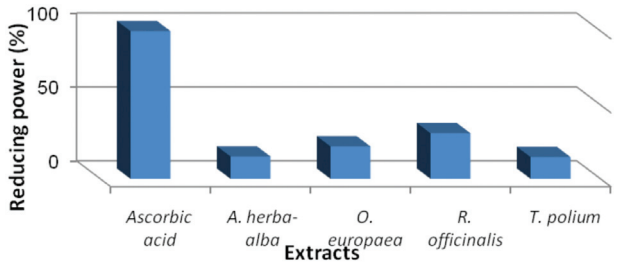
Phenolic compounds are one of the most effective plant secondary metabolites that contribute to the antioxidant activity of plant food and their content is varied with the plant species [26]. The aqueous extract of *O. europaea* was shown to have a phenolic content of 90.402±0.597 mg GAE/100 g. This result was higher than that found by other researchers [15,27], and the different in results may be attributed to the type, cultivar, solvent, and method of leaves extraction. Another reason for different results is the use of high temperature to extract the phenolics, as heat might reduce both the phenolic content and antioxidant activity [28].

Stankovic *et al.* [29] reported that the total phenolic contents of different extract of *T. polium* ranged from 14.57 to 157.84 mg GAE/g, and they found that methanol extract of *T. polium* leaves was the highest (157.84 mg GAE/g), whereas in other extracts, the phenolic compounds contents of *T. polium* were less. The lower phenolic content of *T. polium* in our study is related to the use of hot water to extract phenolics, and water was not the best solvent to extract more phenolics. Studies showed that polar solvents like methanol and ethanol have better extraction for phenolics from plant materials than less-polar solvents like acetone and hexane [30,31]. The solubility of phenolic compounds is different according to the polarity of solvent used [32], and phenolic compounds are more soluble in methanol than aqueous extract; this may explain the low quantity of phenolic compounds obtained from the aqueous extract of *T. polium* in our study.

The extracted amount of phenolic compounds from plant material is highly affected by the solubility of these compounds in the extracting solvent and the polarity of the solvent [25]. Generalic Mekinic *et al.* [33] showed that better phenolic compounds extraction yield of *O. europaea* leaves was obtained by using alcoholic solvents compared with water.

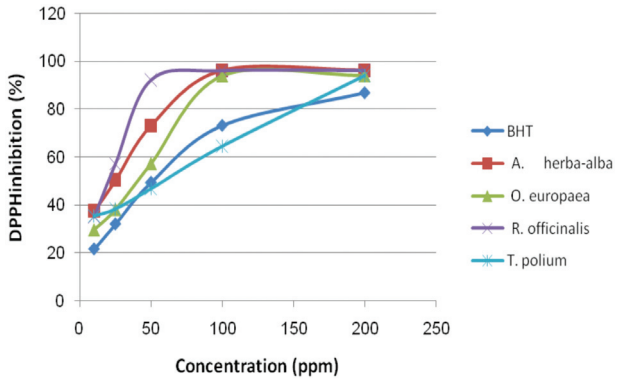
Flavonoids are found in many dietary and medicinal plants with a wide variety of biological activities [34]. Stankovic *et al.* [29] investigated the flavonoidal content in the extracts of *T. polium* and found that the acetone leaves extract had the

**Figure 1**




1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity of aqueous extracts of *A. herba-alba*, *O. europaea*, *R. officinalis*, *T. polium*, and BHT at different concentrations.

**Figure 2**




Reducing power (%) of *A. herba-alba*, *O. europaea*, *R. officinalis*, *T. polium*, and ascorbic acid.

**Table 6 The correlation between total phenolic and flavonoidal contents and 1,1-diphenyl-2-picrylhydrazyl, reducing power, and α-amylase activities**

| | Phenolics at 100 ppm | Flavonoids at 1000 ppm |
|---|---|---|
| DPPH 50 ppm | 0.701* | 0.952** |
| Reducing power (%) | 0.927** | 0.697* |
| α-Amylase inhibition (%) | 0.983** | 0.839** |

DPPH, 1,1-diphenyl-2-picrylhydrazyl. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

highest flavonoids content [139.87 mg retinol equivalent (RE)/g] among other extracts, whereas flavonoids' concentrations of water extract was the lowest. Olive leaves (*O. europaea*) are known for their high flavonoid content owing to the presence of the secoiridoid oleuropein [12,35]. Benhabyles *et al.* [15] found flavonoid content to be 125.13±2.8 mg QE/100 g in *O. europaea* aqueous extract.

Antioxidants are bioactive substances that have the capability to neutralize free radicals. They act at different stages by different mechanisms like hydrogen donation, reducing agent, quenching agent, or chelators of free radicals [36]. DPPH free radical scavenging activity is one of the commonly used methods to measure the antioxidant activity of plants and herbs extract efficiently. The antioxidants existing in the tested plants or herbs extracts have the ability to reduce the DPPH radical stable color (purple) to the nonradical form (DPPH-H) with low intensity purple to yellow color [37].

*R. officinalis* extract had the highest scavenging activity among studied plants extracts (Fig. 1) with complete inhibition at concentration of 50 ppm, whereas BHT was significantly has the lowest scavenging activity (40%) at the same concentration. The potential antioxidant activities of rosemary have been related to its components diterpenes, carnosol and carnosic acid [16,38].

The IC50 values of DPPH free radicals scavenging activities of different studied extracts were in the following increasing order: *R. officinalis*<*A. herba-alba*<*O. europaea*<*T. polium*<BHT. The higher the $IC_{50}$ value of the extract, the lower the antioxidant activity on DPPH radical. All of the aqueous extracts of the studied plants were shown to have antioxidant potential superior to BHT. Ilhami *et al.* [39] found that chloroform extract of *T. polium* is a good source of natural antioxidants comparable to α-tocopherol. Al-Mustafa and Al-Thunibat [40] reported low DPPH radical scavenging activity of the methanol extract of *T. polium*, whereas Brahmi *et al.* [25] showed that *O. europaea* methanol extract has good DPPH scavenging activity.

The antioxidant activity of plant materials has high correlation with their flavonoids content. Stankovic *et al.* [29] investigated the antioxidant activity of different extracts of *T. polium*, and they found that the water extract had the highest DPPH scavenging activity with $IC_{50}$ value of 56.40 μg/ml, which was proportional to our result.

The reducing power of a compound may serve as a significant indicator of its possible antioxidant activity, and the reducing power method is another assay used to determine the antioxidant activity of plants extracts. This method based on the reduction of a ferrous ion analogue to the intensely blue-colored $Fe^{2+}$ complex by antioxidants in an acidic medium [41]. The reducing power of a compound may serve as an indicator of its possible antioxidant activity [42]. However, different polar extracts from the same plant can give different results according to the extracted compounds.

The reducing power activities of the studied plants were in the following decreasing order: Vitamin C>*R. officinalis*>*O. europaea*>*A. herba-alba*>*T. polium*. The reducing power activities of these plants have been related to their phenolic and flavonoidal contents. Salah *et al.* [43] reported that rosemary and olive leaves extract of different varieties had powerful ferrous reducing power but lower than the standard antioxidant ascorbic acid. Molan *et al.* [44] showed that the water extract of *A. herba-alba* had highly significant ferric reducing power activity in comparison with the other studied plant extracts, which was in agreement with our results that directly correlated with its phenolic content results. The ability of plants extracts to reduce $Fe^{3+}$ may be related to the number and position of the hydroxyl group of phenolic compounds and its ability to donate hydrogen [45,46].

α-Amylase is an enzyme secreted from pancreas and required for the breakdown of starches and complex carbohydrates into absorbable monosaccharide [47]. The α-amylase inhibitors obstruct the digestion and absorption of complex carbohydrates and are potentially useful in control of diabetes [48,49].

Our results are in agreement with Kasabri *et al.* [50] in which *T. polium* water extract lacked any positive in-vitro α-amylase inhibitory effect; moreover, Funke and Melzig [51] show high inhibition level of *R. officinalis* (60%). The extent of amylase inhibition correlated with increased concentration of rosmarinic acid [52]. The inhibitory activity of *O. europaea* extract was attributed to both oleuropein and oleanolic acid that are involved in the antidiabetic effect [53]. Some herbs have shown to improve high glucose level in clinical trials, and test results are subject to several factors [54,55]. For example, each herb extract may contain several compounds, and few of them may be effective [56], and the parts of herb and the methods of extraction may yield different active compounds [57].

The present study exhibited significant correlation between the plant phenolics and flavonoidal contents and their antioxidant and α-amylase inhibitory activities. This was in agreement with findings of Miliauskas *et al.* [21] and Silva *et al.* [58] but not in agreement with Javanmardi *et al.* [59] who did not find any kind of correlation. For any plants, the total phenolic and flavonoids contents can be affected by environment and the plant sources; moreover, using different extraction methods reduces the chance of any comparison between studies.

In general, plants that contain large variety of secondary metabolites possess better biological activities; thus, plant bioactivity is dependent on the quantity of its phytochemicals [60]. Antioxidant activities are increased directly with the increase of polyphenol content, and this is mainly because of their redox properties [61]. However, the chemical structure and ability to accept or donate electrons give these compounds the ability to act as antioxidants [62].

## Conclusion

Aqueous extract of *R. officinalis* was the richest in phenolic and flavonoid compounds, and had the highest ability in binding iron and inhibiting DPPH and α-amylase activity. Aqueous extract of *T. polium* was the lowest in phenolic and flavonoid compounds and had the weakest ability in binding iron and inhibiting DPPH and α-amylase activity. Aqueous extracts of *A. herba-alba* and *O. europaea* demonstrated a potent antioxidant activities and α-amylase inhibitory activities.

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Nil.

### Conflicts of interest

There are no conflict of interest.

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