In vitro antioxidant and inhibitory potential of leaf extracts of *Varthemia sericea* against key enzymes linked to type 2 diabetes

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Abstract

Diabetic disease is a chronic metabolic disease characterized by postprandial and fasting hyperglycemia. It is believed that inhibition of α-amylase and α-glucosidase enzymes can be an important strategy in the administration of postprandial hyperglycemia linked to type 2 diabetes. The aim of this study was to evaluate the α-amylase and α-glucosidase inhibitory and antioxidant potential of leaf extracts of *Varthemia sericea* (Batt. et Trab.) Diels. Methanol extract and its fractions, cold water and ethanol extracts of *Varthemia sericea* leaves were initially screened for phytochemical class, antioxidant properties and α-amylase and α-glucosidase inhibitory activities. Furthermore, all extracts of *Varthemia sericea* were found to be potent inhibitors of α-amylase with IC\textsubscript{50} values range of 37.98±2.26 and 119.89±0.89 µg/ml. The methanol extracts of *Varthemia sericea* had both the strongest inhibitory effect against the α-amylase and the α-glucosidase with IC\textsubscript{50} values of 55.63±1.51 and 113.33±1.59 µg/ml, respectively. Besides, all leaf extracts from *Varthemia sericea* showed a good antioxidant activity. Whereas, the observed effects were associated with the determination of phytochemical class such as alkaloids, coumarin, and tannins, etc. Overall, our findings suggest that extract from *Varthemia sericea* leaves may be useful for diabetic care.

Keywords: *Varthemia sericea*, α-Amylase, α-Glucosidase, Antioxidant, Phytochemical screening.

1. Introduction

Diabetes mellitus is a chronic metabolic disease characterized by postprandial and fasting hyperglycemia (Jain and Saraf, 2010). A sudden rise in the blood glucose levels, causing hyperglycemia in type 2 diabetes patients happens due to hydrolysis of starch by pancreatic α-amylase and uptake of glucose by intestinal α-glucosidase (Gray, 1995).

Subsequently, it is assumed that inhibition of these hydrolitic enzymes can significantly diminish the postprandial increase of blood glucose level after different starch diet, and in this way can be a significant technique in the administration of hyperglycemia linked to type 2 diabetes (Kwon et al., 2008).

Currently, there are some antidiabetic drugs, namely, acarbose, continuous use of these latter is often associated with undesirable side effects, such as liver toxicity and adverse gastrointestinal symptoms (van de Laar, 2008; Etxeberria et al., 2012). Experimental data demonstrated that oxidative stress, through the production of reactive oxygenic spieces (ROS), contributes to the progression of diabetes causing damage to insulin action and increased incidence of complications (Giacco and Brownlee, 2010). Natural antioxidants from plants offer a substitute source of dietary ingredients; for example, α-amylase and α-glucosidase inhibitors are considered as one of the effective approaches for regulating type 2 diabetes by controlling glucose uptake and increasing the secretion of insulin hormone (Visweswara et al., 2013).

Existent studies, along with numerous others (Apostolidis and Lee, 2010; Nwosu et al., 2011), add to the increasing body of evidence, that members of the class Asteraceae are an excellent source of enzymes inhibitors.

*Varthemia* genus extracts are known to contain a wide array of bioactive substances with diverse health benefits (Afifi et al., 1997; Al-Dabbas et al., 2006b). It is also known as one of the most common plant species which has long been used as an anti-diabetic herb.

However, reports show that the extracts of *Varthemia* species possessed a strong effect in fasting plasma glucose concentrations reduction in diabetic patients with poor glycemic control. In addition, it has a lowering effect on the blood glucose level in hyperglycemic rats (Afifi et al., 1997).

Due to the increasing attention in the valorization of endemic plants to support their use, the leaf extracts from *Varthemia sericea* (*V. sericea*) were studied herein. *V. sericea* is an endemic plant growing wild in the center of Algerian Sahara. The aqueous extract of *V. sericea* is commonly used as an Algerian folk-medicine for the treatment of gastrointestinal disorders (Hammchich and Maiza, 2006). To the best of our knowledge, this study

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is the first to undertake *V. sericea* leaves extracts that may be useful for diabetic care. To this end, methanol extract and its fractions, cold water, and ethanol based extracts from *V. sericea* leaves were screened for potential α-amylase and α-glucosidase inhibitory activity and antioxidant proprieties.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade and purchased from Sigma Aldrich.

2.2. Preparation of the methanol extract and its fractions

The extractions were carried out according to the method of Markham, (1982). The leaves of *V. sericea* powder were soaked in 75 % aqueous-methanol (V/V) with a ratio of plant material and extracting solvent of 1:10 (W/V), under agitation over three nights. The extract was filtered on filter paper to obtain the first filtrate. This procedure was repeated on the residue using 50% aqueous-methanol (V/V) under agitation to obtain the last filtrate. The first and the last filtrates were combined then the methanol was removed under reduced pressure on a rotavapor below 40 °C.

The methanol extract was subjected to fractionation using liquid extraction; it was successively extracted with different solvents of increasing polarity: Petroleum ether, chloroform, ethyl acetate. The obtained organic layer of each partition was evaporated under reduced pressure on a rotavapor below 40 °C to dryness and to afford petroleum ether, chloroform, ethyl acetate fractions. The methanol and its fractions were stored at 4°C prior to use.

2.3. Cold water and ethanol extracts preparation

30 g of plant material was extracted with 300 ml of 50% aqueous-ethanol (V/V); the same quantity was extracted with 300 ml of distilled water as mentioned in Alimpić et al. (2015a). In both cases, extraction was performed under agitation during 72 h at room temperature.

This process was repeated twice in the same conditions and each of the extracts was gathered and then filtered through a filter paper (Whatman No.1), evaporated under reduced pressure and stored at 4 °C for further experiments.

2.4. Phytochemistry screening

Each extract of the leaves (2–3 mg/ml) of *V. sericea* was subjected to a different primer phytochemical investigation for the identification of various chemical groups (Harborne, 1998), using different tests (Alcaloïdes, steroides, quinoines, coumarins, tannins, anthocyanes, saponins, terpenoids, anthraquinones and cardiac glycosodiques).

2.5. Antioxidant capacity determination

2.5.1. ABTS scavenging activity

The spectrophotometric analysis of 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ABTS⁺ scavenging activity was determined according to the method of Re et al. (1999). The ABTS⁺ was produced by reacting 2 mM ABTS in H₂O with 2.45 mM potassium persulfate [K₂S₂O₈], stored in the dark at room temperature for 16 h. The ABTS⁺ solution was diluted with distilled water to give an absorbance of 0.700 ± 0.025 at 734 nm. 160 μl of this solution was mixed with 40 μl of the sample in methanol and the absorbance was recorded at 734 nm after 10 min. BHT and BHA were used as antioxidant standards.

2.5.2. Galvinoxyl radical (GOR) scavenging assay

Galvinoxyl radical (GOR) scavenging assay was determined according to the method of Shi, (2001) where Galvinoxyl was reduced by hydrogen-donation free radical scavengers. Briefly, 160 μl of GOR (0.1 mM Galvinoxyl) (4 mg in 100 ml methanol) was mixed with 40 μl of the sample in methanol and the absorbance was recorded at 428 nm after 120 min. The given results as an absorbance were compared to those of standards (BHT and BHA).

2.5.3. Hydroxyl (•OH) radical scavenging activity

Hydroxyl radical scavenging activity was measured by the adapted method of Smirnoff and Cumbes (1989). Briefly, 40 μl extract was mixed with 80 μL salicylic acid (3 mM), 24 μL FeSO₄ (8 mM) and 20 μL H₂O₂ (20 mM). After incubation for 30 min at 37°C, 36 μL H₂O was added and the absorbance of the mixtures was measured at 510 nm. Ascorbic acid used as a positive control.

2.5.4. Cupric reducing antioxidant capacity (CUPRAC)

Cu²⁺ ion reduction was assessed by the cupric reducing antioxidant capacity (CUPRAC) method (Apak et al., 2004) by mixing 10 mM of copper chloride [CuCl₂] (50 μl), 7.5 mM of neocuproine in ethanol (50 μl), and 1 M ammonium acetate [CH₃COONH₄] (60 μl) with 40 μl of the sample at diverse concentrations in a microplate of 96 wells. The absorbance was measured at 450 nm after 40 min of incubation. The A₅₅₃ values were calculated from the absorbance curves. BHA and quercetin were used as standards.

2.5.5. O-phenanthroline reduction assay

The reaction mixture consisted of 30 μl O-phenanthroline (0.5% in methanol), 50 μl [FeCl₃] (0.2%), 110 μl methanol, and 10 μl of different concentrations of the extract. The combination was incubated for 20 min at 30°C, the absorbance of an orange-red solution was measured at 510 nm against a reagent blank (50 μl of [FeCl₃] (0.2%) and 30 μl of O-phenanthroline (0.5%) made up to 200 μl with methanol) and the percentage inhibition was calculated. The BHA and BHT were used as a positive standard (Szydłowska-Czerniak et al., 2008).

2.5.6. Ferrous ions chelating activity

The iron-chelating activity of the extracts was measured by the ferrozine method (Hennessy et al., 1984) with some modifications. To 40 μl of each sample (dissolved in methanol) was added 40 μl of methanol and 40 μl 0.2 mM [FeCl₃· 4H₂O]. Ferrozine (80 μl, 0.5 mM) was then added. The combination was agitated vigorously and left at room temperature for 10 min. The absorbance was measured at 593 nm. EDTA was used as a standard.
2.6. Enzyme assay

2.6.1. α-Amylase inhibitory assay

Screenings for α-amylase inhibition by extracts were carried out according to Zengin et al. (2014) with slight modification based on the starch-iodine color method. 25 μl of extracts at different concentration were added to 50 μl solution α-amylase 1U and were incubated for 10 min at 37°C. Then, 50 μl of starch 0.1% was added to each test tube and incubated at 37°C for 10 min. 25 μl of HCl (1M) was added to stop the enzymatic reaction followed by the addition of 100 μl IKI solution. The color change was noted and the absorbance was read at 630 nm. The results were definitive as % inhibition calculated using the formula: %INH=1-(Ac-Ae)/(As-Ab)/(Ac-Ae)

Ae=Absorbance [Starch+IKI+HCl +Vol of the solvent of extract +Vol of Enzyme buffer] Ae=Absorbance [Enzyme + Starch +IKI +HCL + Vol of extract solvent]
As=Absorbance [Enzyme+Extract+ Starch +IKI]
Ab=Absorbance [Extrait+IKI+125µl Buffer]

2.6.2. α-Glucosidase inhibitory assay

10 μl of the sample (extracts or acarbose) solution was added to 120 μl of phosphate buffer (pH 6.9) (0.2 M), 20 μl α-glucosidase (0.5 unit/mL) from Baker’s yeast (in phosphate buffer) was added to each test tube, then 50 μl of 5 mM p-Nitrophenyl-α-D-glucopyranoside solution (prepared in phosphate buffer) was mixed and incubated for 15 min at 37°C. 80 μl of Sodium carbonate solution (0.2 M) was then added. The lecture of absorbances was measured at 405 nm using a microplate reader set to 37°C against a blank (Asghari et al., 2018).

2.7. Statistics Analysis

All data points are mean values ± standard error (SE) of at least three independent experiments. Where appropriate, data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s Multiple Comparison test and Tukey’s Multiple Comparison Test. The software employed for statistical analysis was SPSS IBM 20.0

3. Results

3.1. Determination of phytochemicals classes

The results of the phytochemical screening of leaf extracts from V. sericea were presented in Table 1. Various types of bioactive compounds were detected such as alkaloids, coumarins, and tannins, etc.

### Table 1. Results of phytochemical screening presenting in leaf extracts of Varthemia sericea.

<table>
<thead>
<tr>
<th>Phytochemical Groups</th>
<th>Ethanol</th>
<th>Cold water</th>
<th>Methanol</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Aqueous residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaloïdes</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroides</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarines</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>NT</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Anthocyanes</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+++</td>
<td>++</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cardiaque glycosidiques</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

+++ : Very abundant, ++: Abundant, +: Rare,-: Not detected. NT: Not tested.

Furthermore, the hydroxyl radical scavenging activity was increased significantly after hydrolysis and the highest activity was observed with IC50 values (288.08±2.08 and 360.40±1.57μg/ml) for ethanol and methanol extracts, respectively (Table 2). These results were compared against ascorbic acid (IC50= 13.86 ± 0.47 μg/ml). Moderate activity was observed with cold water extract, aqueous residue and chloroform fraction (IC50= 523.70±0.96, 638.06±1.69 and 696.61±2.18 μg/ml, respectively).

In the same context, the methanol extract showed a potential O-phenanthroline reduction with A0.5 of 117.63±1.97 μg/ml followed by chloroform, ethanol, ethyl acetate and aqueous residue 163.55±2.25, 166.85±1.4, 182.48±1.10 and 184.22±2.78μg/ml, respectively. Cold water and petroleum ether had a slight effect with A0.5 438.26±2.17 and 501.5±0.57 μg/ml, respectively, compared to the standards (BHA and BHT) where they exhibited a strong effect against O-phenanthroline assay with A0.5 0.93±0.07 and 2.24±0.17μg/ml, respectively.

Based on the outcomes, the ethyl acetate fraction exhibited a high potential effect against ABTS scavenging assay followed by methanol and ethanol extracts with IC50 of 53.72 ± 2.22, 83.22 ± 0.33 and 87.20 ± 1.33 μg/ml, respectively.

For the Galvinoxyl radical (GOR) aqueous residue fraction exhibited a scavenging capacity with an IC50 value of 32.44 ± 4.73 μg/ml, while ethyl acetate fraction, methanol extract, ethanol extract and chloroform fraction exhibited a similar scavenging activity with IC50 values of 137.96 ± 3.06, 137.38 ± 2.47, 146.62 ± 4.01 and 156.41 ± 2.42 μg/ml, respectively. Lowest values were recorded with petroleum ether fraction and cold water extract that exhibited close capacities with no significant difference (P ≤ 0.05).
Table 2. Antioxidant activity of leaf extracts of *Varthemia sericea* (IC50 and A0.5) µg/ml ±SD.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Radical scavenging activity IC50 (µg/mL)</th>
<th>Reducing power A0.5 (µg/mL)</th>
<th>Metal chelating assays IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABTS</td>
<td>GOR</td>
<td>OH</td>
</tr>
<tr>
<td>Ethanol</td>
<td>87.20±1.33d</td>
<td>156.41±2.42d</td>
<td>288.08±2.08e</td>
</tr>
<tr>
<td>Cold Water</td>
<td>150.30±1.62c</td>
<td>342.56±4.22c</td>
<td>523.70±0.96d</td>
</tr>
<tr>
<td>Methanol</td>
<td>83.22±0.33c</td>
<td>137.38±2.47c</td>
<td>360.40±1.57d</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>155.07±0.63c</td>
<td>715.68±1.97a</td>
<td>Na</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>153.57±1.63c</td>
<td>146.62±4.01a</td>
<td>696.61±2.18a</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>53.72±2.22c</td>
<td>137.96±3.06e</td>
<td>Na</td>
</tr>
<tr>
<td>Aqueous Residue</td>
<td>279.10±2.24c</td>
<td>32.44±4.73f</td>
<td>638.06±1.69b</td>
</tr>
<tr>
<td>Standards</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHA</td>
<td>1.81±0.10j</td>
<td>5.38±0.06i</td>
<td>NT</td>
</tr>
<tr>
<td>BHT</td>
<td>1.29±0.30h</td>
<td>3.32±0.18h</td>
<td>NT</td>
</tr>
<tr>
<td>EDTA</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>NT</td>
<td>NT</td>
<td>13.86±0.47f</td>
</tr>
</tbody>
</table>

• Na: No activity (>800 µg/ml), NT: Not tested. Values expressed were means ± S.D. of three parallel measurements (P ≤ 0.05).
• Results with different superscript letters are significantly different (P ≤ 0.05) and the decrease order (a>b>c>d>e>f>j>..).
• (ABTS+ radical scavenging, Galvinoxyl radical (GOR) scavenging antioxidant capacity assay, Hydroxyl Radical Scavenging Assay, Cupric reducing antioxidant capacity (CUPRAC), O-phenanthroline reduction assay (O-phen), Ferrous iron chelating (FIC) assay.

In addition, the capacity of different extracts to reduce copper ions property was assessed using CUPRAC assay. The different extracts and fractions exhibited an interesting reduction of copper ions at the tested concentrations. The aqueous residue fraction was the most efficient in the reduction of copper ions, followed with the ethyl acetate fraction, methanol, and ethanol extracts with *A0.5* values of 45.99 ± 2.34, 52.17 ± 1.04, 66.39 ± 0.89 and 94.39±1.47 µg/ml respectively. Chloroform and petroleum ether fractions, as well as the cold water extract, exhibited the lowest reducing capacity of copper ions.

Moreover, all extracts did not show any capacity to chelate iron ions (IC50>800µg/ml). Interestingly, EDTA used as standard showed a strong chelating property (IC50 = 12.11 ± 0.32 µg/ml) (P ≤ 0.05).

These results show that the leaf extracts of *V. sericea* exhibited a dose-dependent antioxidant activity.

3.3. Determination of enzymes inhibitory activity of leaf extract from *Varthemia sericea*

The extracts of interest, methanol extract and its fractions, cold water, and ethanol extracts of the leaf of *V. sericea* were examined at lower concentrations (10-500µg/ml) and initially screened for α-amylase and α-glucosidase inhibitory effects.

The results of the determination of α-amylase and α-glucosidase inhibitory by the exhibition of *V. sericea* leaves extracts were presented in Figure 1, and compared to the quercetin and ascarbose as positive standards.

3.3.1. Determination of α-amylase inhibitory activity

The effect of the inhibitory enzyme showed that all the extracts exhibited a significant potential activity more than 50% of inhibition against α-amylase, at a concentration of 125 µg/ml (Figure 1. A).
Where the aqueous residue fraction had the best inhibition with amylase 62.70 %, followed by the ethyl acetate and methanol extracts with 58.13 and 57.52 %, respectively.

The IC50 values were obtained from the dose–response curves. The IC50 of the inhibitory effect of extracts of V. sericea was significantly stronger than the acarbose, represented in Figure 1. B. As a result, ethyl acetate, residue aqueous, cold water and methanol extracts acted as α-amylase inhibitory effect with IC50 of 37.98±2.26, 41.87±1.46, 50.54±0.68 and 55.63±1.51 µg/ml, respectively. Followed by petroleum ether, chloroform, ethanol extracts with IC50 of 95.89±0.89, 99.79±0.46 and 119.89±0.89 µg/ml, respectively. We can say that all leaf extracts from V. sericea showed a strong effect compared to the acarbose (IC50= 3650.93±10.70 µg/ml).

3.3.2. Determination of α-glucosidase inhibitory activity

The results of the α-glucosidase inhibitory effect of leaf extracts of V. sericea are mentioned in Table 1. At a concentration of 125 µg/ml, the methanol extract had a percentage inhibition of 54.90 % followed by ethanol and cold water extract of 25.13 and 39.56 %, respectively. These results were lower than the quercetin (IC 50=4.26±0.24 µg/ml). However, the methanol and cold water extracts showed a strong effect compared to the acarbose (IC50= 275.43±1.59 µg/ml) and with amylase 62.70 %, at a concentration of 312.5 µg/ml, which is more effective than acarbose (IC50= 3650.93±10.70 µg/ml). We can say that all leaf extracts from V. sericea showed a strong effect compared to the acarbose (IC50= 3650.93±10.70 µg/ml).

For the inhibitory of α-glucosidase, the methanol extract inhibited the 50% of the α-glucosidase enzyme at a concentration of 113.33±1.59 µg/ml, which is more effective than acarbose and 62.70 %, at a concentration of 312.5 µg/ml.

Based on the outcomes, the methanol extract from leaves of V. sericea had a dual inhibitory effect against the two enzymes, which should have a specific inhibitor compound for each enzyme.

4. Discussion

V. sericea is a medicinal plant from the pharmacopeia of the central Sahara of Algeria, used to treat a wide range of diseases. The leaves of this plant have been reported to possess antispasmodic and analgesic effects used as a therapy in traditional medicine (Hamminehe and Maiza, 2006).

To our knowledge, no detailed study of V. sericea has been delivered earlier. In the present study, we reported the inhibition effect of different leaf extracts of V. sericea on the two key enzymes linked to type 2 diabetes and the antioxidant activities due to the presence of bioactive molecules.

Qualitative phytochemical screening of different chemical classes in extracts of V. sericea revealed the presence of the alkaloids, tannins, and coumarin, which contributes to the antioxidant and anti-diabetic activities of the plant (Table 1). We can explain the presence, the absence, and the abundant or a rare presence of that chemical class by the solvent system extract which was verified by Ogbaru et al. (2014).

Previous studies on the chemistry of Varthemia species, (Jasonia = Varthemia), Jasonia glutinosa, Jasonia berosa, Jasonia montana, and Jasonia candidas, had shown the presence of different phytomolecules such as sesquiterpenes and sesquiterpene lactone derivatives 1 eudesmanoic acids, eudesmanolides,2 guaianolides and pseudoguaianolides, 2 together with various polymethoxylated flavonoids and coumarins (De Pasceau et al., 1980; Ahmed et al., 1993; 1994). Jassonia montana is considered as a rich plant in polyphenols which are used as antioxidants (Al-Howiriny et al., 2005).

The existence of secondary metabolites such as the flavonoids adds value to this herb since they are a group of compounds that are known to exhibit antioxidant, and thereby anti-diabetic activities (Halliwell and Gutteridge, 1999; Ng et al., 2000), which are caused by exposure to oxidative stress (Liu, 2003; Caillet et al., 2006).

Subsequently, the leaf extracts of V. sericea were evaluated for their biological potentialities.

It is significant to use different assays to take in consideration the composition of extracts which act through several mechanisms like the inhibition of chain initiation, binding of transition metal ion catalysts, breakdown of peroxides, avoidance of continued hydrogen abstraction, reductive ability and radical quenching (Li et al., 2008).

For this reason, the antioxidant ability of V. sericea was evaluated using six complementary -in vitro- tests: free-radical scavenging (ABTS, OR, and OH), reducing power (CUPRAC, phenanthroline) and metal chelating assays.

The results are summarized in Table 2. Free radical scavenging is thought to be one of the main mechanisms exhibited by antioxidants to delay oxidative processes. ABTS is stable free radical used to determine the ability of antioxidants to donate a hydrogen atom by converting it to the non-radical species (Alam et al., 2013). One mechanism of protecting consequence may be radical-scavenging which is via offering hydrogen atom (H·), donating electron (e·). Its antioxidant ability can be mainly attributed to the existence of phytochemicals such as flavonoids or total phenolic (Li et al., 2013).

The ethanol and aqueous extracts from the aerial part of V. iphionoides showed a pronounced 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, with an inhibition of about 90%, at a concentration of 100 µg/ml (Al-Debbas et al., 2006a).

In a study carried out by Kaiser et al. (2011), the aqueous extract of Ficus racemosa (Family of Moraceae) seed revealed a strong free radical scavenging activity having an IC 50 value of 22.10 µg/ml.

As a reducer agent, CUPRAC and the red O-phenanthroline, is widely applied in the classical spectrophotometric method for the determination of iron. The reduction can be used as an indicator of electron-donating activity, which reflects an important mechanism of antioxidant action (Hajji et al., 2019).

However, the chelation of ferrous ions can be due to the presence of the nitrogen groups explaining the activity of alkaloids (Hennessy et al., 1984).

The hydroxyl groups in polyphenols are responsible for their antioxidant properties, which is manifested by canceling the effect of free radicals such as peroxo, oxo, and hydroxo (Teodora et al., 2020). The results of several recent studies have demonstrated that the hydroxyl groups in polyphenolic compounds may perform a crucial function in promoting inhibitory activity (Stern et al., 1996; Kim et al., 2008).

As it has been suggested, the phenolic content of plant materials is correlated with their antioxidant activity; the
antioxidant potentials of natural compounds are referred to as their capacity to scavenging radicals and by chelating metal ions via functional groupement of their structure. Besides, it has been suggested that the antioxidant and lipid peroxidation-inhibiting potential of flavonoid predominantly resides in the radical-scavenging capacity rather than the chelation of metals (Iriga et al., 1987; Ratty and Das, 1988).

Solvents are a key step for extracting antioxidants from natural sources. Ethanol was with the highest frequency as a solvent for extraction purposes. These results suggested that the phenolic compounds that existed in these extracts are mainly flavonoids with different methoxyl and hydroxyl substitutions. The hydroxyl substitution is mainly responsible for hydrogen donations to the free radicals (Alam et al., 2013).

Antioxidant activity contributes a part in the administration of diabetes mellitus (Raphael et al., 2002). Some plant species have been reported to account for both antidiabetic activities as well as antioxidant activity. Phenolic from tea is found to manage both α-amylase and sucrase, which have an antioxidant effect (Matsumoto et al., 1993; Tchinda et al., 2008).

Antioxidants from plants offer a substitute source of dietary ingredients. For example, α-amylase, α-glucosidase inhibitors are considered as one of the effective measures for regulating type 2 diabetes by controlling glucose uptake and increasing the secretion of insulin hormone (Kim et al., 2009).

Enzyme inhibition is a common and important method for the discovery of new drugs and the treatment of public human health disorders. There are several enzymes whose inhibition is considered as a target for the treatment or prevention of related diseases (Asghari et al., 2018), including glucosidase and amylase (Diabetes Mellitus).

According to numerous in vitro studies, inhibition of α-amylase and α-glucosidase is believed to be one of the most effective approaches for diabetes care (Etcheberria et al., 2012; van de Laar, 2008).

Based on the finding in Figure 1, the methanol extract of V. sericea exhibited both potential inhibitory effect against the two enzymes tested (α-amylase and α-glucosidase). Interestingly, all extracts played a strong inhibitory effect against α-amylase.

The phytochemical such as polyphenol compounds in plants inhibit the activities of carbohydrate hydrolyzing enzymes, such as α-amylase and α-glucosidase, due to their ability to bind with proteins (Mai et al., 2007).

The α-amylase inhibitors are also called starch blockers, as they contain substances that prevent dietary starch from being absorbed into the body system, which can be convenient within the management of diabetes. α-amylase may exert a blood-glucose-lowering effect through inhibition of salivary and pancreatic amylase (Kazeem et al., 2013; Harrison et al., 2014).

In a study carried out by Al-Dabbas et al. (2006b), two methods were tested, the ethanol and water extracts had a weak but substantial inhibitory effect on α-amylase in the iodine-starch assay, whereas they had a pronounced effect with inhibition of about 70% at a concentration of 200 mg/ml in the 2-chloro-4-nitrophenyl alpha-maltotrioside (CNP-G3) assay. The different results of α-amylase inhibition by the two methods might be attributable to the different substrates used. As a result, the cleavage of short-chain substrates, such as CNP-G3 was reduced by inhibitory substances to a larger extent than with longer sequence substrates in the Iodo-starch method, and this probably clarifies the complete inhibition of the α-amylase activity by the CNP-G3 method even at a low concentration (100 mM) (Al-Dabbas et al., 2006b).

In other veins, α-glucosidase inhibitors are suppressor of postprandial hyperglycaemia in diabetic patients by inhibiting the activity of α-glucosidase in the intestine, which reduces glucose absorption by delaying carbohydrate digestion and increases digestion time (Johnston et al., 2010).

The genus of Vathemia (also named Jasiona) has long been used as an anti-diabetic herb, and it has a lowering effect on the blood glucose level in hyperglycemic rats (Al-Dabbas et al., 2006b).

Reports show that Vathemia iphionoides extract possessed a moderate effect in fasting plasma glucose concentrations reduction in diabetic patients with poor glycemic control (Abu-Zaifon et al., 2018).

Results of the study carried out by Alfii and colleagues (1997) revealed the decreasing of blood glucose level by Vathemia iphionoides essential oil, maybe due to its numerous bioactive compounds such as phenols, flavonoids, saponins, triterpenoids and tannins; these compounds may play their role in hypoglycemic effects by increasing release and decreasing gluconon’s secretion, decreasing insulin resistance, slowing the absorption of glucose or by reducing hepatic glucose production (Abu-Zaifon et al., 2018).

Numerous studies on plant extracts of Chrysanthemum genus (Asteraceae) that may contribute to diabetes management have focused on the inhibition of α-glucosidase which catalyzes carbohydrate consumption into glucose (Yang et al., 2011; Thi Luyen et al., 2013; Ben Sassi et al., 2018).

Indeed, these findings demonstrate that the leaf extracts are able to inhibit the oxidative reactions by free radical scavenging and by acting as reducing and chelating power agents as well as the α -amylase and α-glucosidase inhibitory activities. These therapeutic promises can be ascribed to their phenolic constituents; although, other phytochemicals could have acted synergistically (Ademiluyi et al., 2015).

5. Conclusion

To sum up, the results showed that all leaf extract of V. sericea presented different phytochemical classes such as alkaloid, tannins, and coumarin, well known for their antioxidants and anti-diabetic effects. These leaf extracts exhibited potential inhibition effects against the two key enzymes linked to type 2 diabetes and antioxidant activities. Further works are needed to isolate and identify the bioactive molecules from the leaves of V. sericea.

Conflicts of interest

None.

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