We present here the evaluation of acetylcholinesterase (AChE) inhibitory and antioxidant activities of the aqueous celery in patients with Alzheimer’s disease. It was observed like increasing patients with Alzheimer’s disease and formation of neurofibrillary tangles in countries where herbs and remedies which are significantly more in approaches.

The cell death report that deficit in cholinergic system is responsible for cognitive decline and memory loss in patients with Alzheimer’s disease. Various pharmacologic approaches are developed for the treatment of Alzheimer’s disease. The leading edge therapies of Alzheimer’s disease are approved drugs; Acetylcholinesterase inhibitors and NMDA receptor antagonist. The experimental therapies are mostly disease modifying and have neuroprotective approaches. Antioxidants, anti inflammatory agents and statins help by preventing oxidation and inflammation of neurons (Prema, 2010).

Alzheimer’s disease is characterized by atrophy of cerebral cortex and loss of hippocampal and neocortical neurons. The pathological hallmark of Alzheimer’s disease is widespread neuritic plaques which are accumulations of amyloid beta protein and neurofibrillary tangles. Studies report that deficit in cholinergic system is responsible for cognitive decline and memory loss in patients with Alzheimer’s disease. Various pharmacologic approaches are developed for the treatment of Alzheimer’s disease. The leading edge therapies of Alzheimer’s disease are approved drugs; Acetylcholinesterase inhibitors and NMDA receptor antagonist. The experimental therapies are mostly disease modifying and have neuroprotective approaches. Antioxidants, anti inflammatory agents and statins help by preventing oxidation and inflammation of neurons (Prema, 2010).

Oxidative stress is present within the brains of patients with Alzheimer disease. Treatment with antioxidants is a promising approach for reducing disease progression. Recent research has found a link between antioxidant intake and reduced incidence of dementia (Grundman et al., 2002). A review of antioxidants has suggested agents like aged garlic extract, curcumin, melatonin, resveratrol, Ginkgo biloba extract, green tea and Vitamin C and E in patients with Alzheimer’s disease (Frank et al., 2005). Some antioxidants showing promise is Vitamin E or Alpha tocopherol. A study compared the effect of Vitamin E and Selegiline alone, together and placebo in patients with Alzheimer’s disease. It was observed that delay to one of the primary outcomes like time to death, confinement or development of severe dementia was significantly more in Vitamin E groups as compared to placebo Sano et al., 1997). Therefore, many practitioners have added Vitamin E supplements to the standard.

### Keywords:
- Acetylcholinesterase, Alzheimer, antioxidant, anti-lipidperoxidation, medicinal plants

### Introduction

The investigation of plants used as remedies in traditional folk medicine can be as useful tool to identify several biologically active molecules from the 250 000 higher plant species (Testai, et al., 2002). The modern pharmacopoeia still contains at least 25% drug derived from plants and many others, which are synthetic analogues of compounds isolated from plants. Despite the availability of different approaches for the discovery of therapeutics, natural plant products remain as one of the best reservoirs of new structural types (Mahomoodally, et al., 2012). The use of herbs and medicinal plant products has become a prevailing phenomenon over the past two decades in several countries where herbs and phytomedicines (herbal remedies) has the fastest growing segments in retail pharmacies and supermarkets.

Alzheimer’s disease is a devastating neurodegenerative disorder manifested by deterioration in memory and cognition, impairment in performing activities of daily living, and many behavioral and neuropsychiatric illnesses. The pathological hallmark of Alzheimer’s disease is widespread neuritic plaques which are accumulations of amyloid beta protein and neurofibrillary tangles. Studies report that deficit in cholinergic system is responsible for cognitive decline and memory loss in patients with Alzheimer’s disease. Various pharmacologic approaches are developed for the treatment of Alzheimer’s disease. The leading edge therapies of Alzheimer’s disease are approved drugs; Acetylcholinesterase inhibitors and NMDA receptor antagonist. The experimental therapies are mostly disease modifying and have neuroprotective approaches. Antioxidants, anti inflammatory agents and statins help by preventing oxidation and inflammation of neurons (Prema, 2010).

### Abstract

**Background:** Numerous plants in traditional practices of medicine have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer’s disease (AD) and other memory related disorders.

**Materials and Methods:** We present here the evaluation of acetylcholinesterase (AChE) inhibitory and antioxidant activities of the aqueous methanol extracts of five traditional medicinal plants. *Citrullus colocynthis, Emex spinosa, Rhazya stricta, Scrophularia hypericifolia and Caylusea hexagyna* extracts were tested for their acetylcholinesterase inhibitory effect and their antioxidant effect at different concentrations.

**Results:** *Citrullus colocynthis and Emex spinosa* inhibited acetylthiocholinesterase at 400 µg/ ml by 83.54 and 81.92%. *Emex spinosa and Scrophularia hypericifolia* produced the maximum effect as DPPH radical scavenger (IC$_{50}$= 10.89 and 11.88µg/ml, respectively). *Scrophularia hypericifolia* showed the highest effect as superoxide radical scavenger (IC$_{50}$= 20.83 µg/ ml) also it produced the highest ability to scavenge hydrogenperoxide radicals (IC$_{50}$= 8.66 µg/ ml) while *Emex spinosa* and *Caylusea hexagyna* showed least IC$_{50}$ for ferrous ion chelation (IC$_{50}$=15 µg/ ml) with powerful reduction capability.

**Conclusion:** The determined antioxidant properties magnified the total antioxidant effect determined by ABTS assay that completely inhibited lipidperoxidation at 200 µg/ ml.

### Keywords:
- Acetylcholinesterase, Alzheimer, antioxidant, anti-lipidperoxidation, medicinal plants
treatment regimen of Alzheimer’s disease. Through this point of view and as part of our continuous research is to study the folkloric use of medicinal plants and herb (Shahat et al., 2013; Kuete et al., 2013; Shahat et al., 2014), the present study was planned to evaluate antioxidant and acetylcholinesterase inhibitory activities of five traditionally medicinal wild plant extracts with known medicinal properties collected from Tanhat, Saudi Arabia.

Materials and Methods

Chemicals

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, potassium ferricyanide, polyoxyethylene (20) sorbitan monolaurate (Tweens), Ascorbic acid (Vc), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), eserine hemisulfate salt. [5,5-dithiobis [2-nitrobenzoic acid] DTNB, sodium dihydrogen orthophosphate (NaH₂PO₄•2H₂O), disodium hydrogen phosphate (Na₂HPO₄•2H₂O), ABTS (2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid), diammonium salt, Tris-HCl buffer, acetylthiocholine iodide and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich, Germany.

Plant materials and extract Preparation

All the plants (Table 1) were collected from the Tanhat protected area, Saudi Arabia in April 2012. The plants were identified by the Plants Taxonomist at the Herbarium Unit. The voucher specimens have been deposited at the Herbarium of the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Sample preparation

The plants were collected and dried under shade. The dried samples were powdered and used for solvent extraction. For extract preparation about 75g of dried sample was extracted twice with 300 mL of 80% methanol. The extracts were according to (Shahat et al, 2014).

Table 1: Medicinal plants used in the present study

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant species (Voucher specimen)</th>
<th>Family</th>
<th>Traditional use</th>
<th>Yield in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Citrullus colocynthis (15954)</td>
<td>Cucurbitaceae</td>
<td>Treat constipation, diabetes, edema, fever, jaundice, bacterial infections as well as cancer (Khall et al., 2010).</td>
<td>14.2 %</td>
</tr>
<tr>
<td>2</td>
<td>Emex spinosa (15955)</td>
<td>Polygonaceae</td>
<td>Purgative, diuretic, a remedy for stomach disorders, dyspepsia and colic (Ahmed and Ibrahim, 2011).</td>
<td>16.8 %</td>
</tr>
<tr>
<td>3</td>
<td>Rhazya strict (15957)</td>
<td>Apocynaceae</td>
<td>Diabetes mellitus, fever, sore throat, inflammatory conditions and helminthiasis (Ali Gonemi, 1992).</td>
<td>22 %</td>
</tr>
<tr>
<td>4</td>
<td>Scrophularia hypericifolia (15958)</td>
<td>Scrophulariaceae</td>
<td>Antipyretic, febrifuge and anti-bacter i al, as a remedy for evening fever, mouth dryness, constipation, prurigo, furuncul osis, sore throat, ulcerous stomatitis, tonsil lits and in the treatment of cancer (WHO and IMM, 1990: Nguyen et al. 2005).</td>
<td>12.12 %</td>
</tr>
<tr>
<td>5</td>
<td>Caylusea hexagyna (15959)</td>
<td>Resedaceae</td>
<td>Anticancer (melanoma cell lines) (Sathiyamoorthy et al., 1999).</td>
<td>13.04 %</td>
</tr>
</tbody>
</table>

Acetylcholinesterase inhibition assay

The enzymatic activity was measured using an adaptation of the method described in Ingkanin et al. (2003). 500 µl of DTNB (3 mM), 100 µl of AChI (15 mM), 275 µl of Tris–HCl buffer (50 mM, pH 8 ) and 100 µl of each plant extract at different concentrations ( 25, 100 and 400 µg/ml) were dissolved in ethanol and were added to a 1 ml cuvette, this cuvette was used as blank. In the reaction cuvette, 25 µl of buffer were replaced by the same volume of an enzyme solution containing 0.28 U/ml. The reaction was monitored for 5 min at 405 nm. Velocities of reaction were calculated. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer instead of inhibitor (extract). Inhibitory activity was calculated from 100subtracted by the percentage of enzyme activity. Data presented here are the average of three replicates. Eserine hemisulfate salt was used as positive control and it was tested at different concentration different from samples. The tested eserine concentrations were 0.1, 0.4 and 1.6 µg/ml to calculate its IC₅₀.

Antioxidant properties

Free radical scavenging activity

The free radical scavenging effect of Citrullus colocynthis, Emex spinosa, Rhazya strict, Scrophularia hypericifolia and Caylusea hexagynaxextracts was measured by 1,1-diphenyl-2-picryl- hydrazil (DPPH) using the method of Yamaguchi et al (1998). Briefly, 0.1 mM solution of DPPH in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml of AD polysaccharide and standards solution at different doses (25, 50, 100, 200 and 400 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance
was measured at 517nm in a spectrophotometer (Jasco V630, serial no. C317961148). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH’ radical concentration in the reaction medium was calculated from the following equation: DPPH’ scavenging effect (\%\%) = 100 – [(A0−A1)/A0] × 100, Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample of extracts (Oktay et al., 2003).

### Reduction capability

The reducing power of plant extracts was determined according to the method of Oyaizu (1986). The different doses of plant extracts (25, 50, 100, 200 and 400μg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 10000 x g (MSE Mistrall 2000, UK, and Serial No.: S693/02/444). The upper layer of solution (2.5ml) was mixed with methanol (2.5ml) and FeCl₃ (0.5ml, 0.1%), and the absorbance was measured at 700nm in a spectrophotometer. Vitamin C acid was used as a control. Higher absorbance of the reaction mixture indicated greater reducing power.

### Metal chelating activity

The chelating of ferrous ions by plant extracts and standard was estimated by the method of Dinis et al. (1994). Briefly, extract and standard (25, 50, 100, 200 and 400μg/ml) were added to a solution of 2mM FeCl₃ (0.05 ml). The reaction was initiated by the addition of 5mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe³⁺ complex formation was given by the formula: Inhibition (%) = [(A0−A1)/A0] × 100, Where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample of polysaccharide and standards. The control contains FeCl₃ and ferrozine (Gülçin et al., 2003a).

### Superoxide anion scavenging activity

Superoxide radicals are generated in phenazine methosulphate (PMS)-nicotinamide adenine di nucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3ml of Tris-HCl buffer (16mM, pH 8.0) containing 1ml of NBT (30μM) solution, 1ml NADH (78μM) solution and 1ml sample solution of extracts at different concentrations were mixed. The reaction was started by adding 1ml of PMS solution (10μM) to the mixture. The reaction mixture was incubated at 25°C for 5min, and the absorbance at 560nm in a spectrophotometer was measured against blank samples. Vitamin C acid was used as controls. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula: Inhibition % = [(A0−A1)/A0] × 100, Where A0 was the absorbance of the control (l-Ascorbic acid), and A1 was the absorbance of extract or standard.

### Scavenging of hydrogen peroxide

The ability of the extracts and standard to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts and standard (25, 50, 100, 200 and 400 μg/ml) in methanol were added to a hydrogen peroxide solution (0.6ml, 40mM). Absorbance of hydrogen peroxide at 230nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of extract and standard compound was calculated using the following equation: H₂O₂ (\%) = [(A0−A1)/A0] × 100, Where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample of extract and standard (Gülçin et al., 2003b).

### Total antioxidant capacity

Total antioxidant activity was measured according to the method described by Miller and Rice-Evans (1997) & Arnao et al. (2001). Exactly 0.2ml of peroxidase (4.4 units/ ml). 0.2ml of H₂O₂ (50 μM), 0.2 ml of ABTS (2,2-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt, 100μM) and 1ml methanol were mixed, and were kept in the dark for 1 hour to form a bluish green complex after adding of 1ml plant extracts of different concentrations or VC was used as a control. All were tested in triplicates. The absorbance at 734nm was measured to represent the total antioxidant capacity and then was calculated as follows: Total antioxidant activity (\%) = [1− (A_{sample}/A_{control})] × 100.

### Lipid Peroxidation-Ammonium Thiocyanate

The antioxidant activity of plant extracts and standards was determined according to the method of with some modifications (Gülçin et al 2002). A pre emulsion was prepared by mixing 175 μg Tween 20, 155 μl linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). A 1 mL of sample in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7.8) and distilled water (pH7.9). The mixed solutions of all samples (21mL) were incubated in screw cap-tubes under dark conditions at 40°C at certain time intervals. To 0.1mL of this mixture was pipeted and added with 9.7mL of 75% and 0.1mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02M ferrous chloride in 3.5% HCl was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer. Antioxidant assay of VC was also determined for comparison. All test data was the average of three replicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation: Inhibition (%) = [(A₀−A₁)/A₀] × 100, Where A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of extracts or standard compounds.
Conventional statistical methods were used to calculate means and standard deviations of three replicates were carried out with the different methods. Analysis of variance (ANOVA) was applied followed with Post Hoc test to determine differences ($p<0.05$ and 0.01).

**Table 2:** Acetylcholinesterase inhibitory effect of plant extracts at different concentrations

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Percentage of inhibition (%)</th>
<th>25 μg/ ml</th>
<th>100 μg/ ml</th>
<th>400 μg/ ml</th>
<th>IC$_{50}$ (μg/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrullus colocynthis</td>
<td></td>
<td>9.11±1.42</td>
<td>36.76±2.10</td>
<td>83.54±2.10</td>
<td>131.02</td>
</tr>
<tr>
<td>Emex spinosa</td>
<td></td>
<td>7.92±0.99</td>
<td>33.63±1.87</td>
<td>81.92±2.04</td>
<td>142.38</td>
</tr>
<tr>
<td>Rhazya stricta</td>
<td></td>
<td>1.67±0.24</td>
<td>25.66±1.84</td>
<td>76.36±1.61</td>
<td>191.44</td>
</tr>
<tr>
<td>Scrophularia hypericifolia</td>
<td></td>
<td>3.54±0.37</td>
<td>5.99±0.77</td>
<td>15.79±1.70</td>
<td>310.03</td>
</tr>
<tr>
<td>Caylusea hexagyna</td>
<td></td>
<td>4.00±0.25</td>
<td>11.32±1.03</td>
<td>18.48±0.97</td>
<td>264.56</td>
</tr>
<tr>
<td>Eserine hemisulphate (positive control)</td>
<td></td>
<td>It reproduced IC$_{50}$=0.03μg/ ml</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

**Acetylcholinesterase inhibitory effect of plant extracts**

*Citrullus colocynthis, Emex spinosa, Rhazya stricta, Scrophularia hypericifolia and Caylusea hexagyna* extracts were tested for their acetylcholinesterase inhibitory effect at different concentrations. The percent inhibition data for plant extracts is presented in Table 2. Data are presented as mean of triplicates ± standard deviation. Data were analyzed by ANOVA one way followed with Post Hoc for multiple comparisons. Groups have the same letter have no significant difference between them. IC$_{50}$ is a concentration that reproduces 50% inhibition. These presented data showed the moderate effect of *Citrullus colocynthis, Emex spinosa, Rhazya stricta*. They inhibited acetylthiocholine iodide hydrolysis at 400 μg/ ml by 83.54%, 81.92% and 76.36%, respectively. However, the other two plant extracts, *Scrophularia hypericifolia* and *Caylusea hexagyna*, showed weak activity (<50% inhibition) against acetylcholinesterase. Data in the same table showed the gradual increment in a linear correlation between plant extract concentration and inhibition percentage.

**Scavenging effect of plant extracts against different radicals and ferrous metal ion (Table: 3)**

*Citrullus colocynthis, Emex spinosa, Rhazya stricta, Scrophularia hypericifolia and Caylusea hexagyna* extracts scavenged 1,1-diphenyl-2-picryl-hydrazil (DPPH•) radicals by more than VC, reference compound. *Emex spinosa* and *Scrophularia hypericifolia* produced the maximum effect than the other extracts (IC$_{50}$= 10.89 and 11.88 μg/ ml, respectively) followed with *Citrullus colocynthis, Rhazya stricta* and *Caylusea hexagyna* by nearly the same IC$_{50}$.

**Table 3:** Scavenging effect of plant extracts against different radicals and metal ion chelation

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>IC$_{50}$ values (μg/ ml)</th>
<th>DPPH</th>
<th>Superoxide radical</th>
<th>H$_2$O$_2$</th>
<th>Ferrous ion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPPH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrullus colocynthis</td>
<td>12.83±1.01</td>
<td>62.51±0.05</td>
<td>25.99±1.61</td>
<td>16.67±1.36</td>
<td></td>
</tr>
<tr>
<td>Emex spinosa</td>
<td>10.89±0.98</td>
<td>68.18±0.13</td>
<td>28.34±1.22</td>
<td>15.99±1.42</td>
<td></td>
</tr>
<tr>
<td>Rhazya stricta</td>
<td>12.80±1.13</td>
<td>37.51±0.24</td>
<td>15.59±1.47</td>
<td>18.60±0.97</td>
<td></td>
</tr>
<tr>
<td>Scrophularia hypericifolia</td>
<td>11.88±1.34</td>
<td>20.83±0.37</td>
<td>8.66±1.68</td>
<td>20.51±1.87</td>
<td></td>
</tr>
<tr>
<td>Caylusea hexagyna</td>
<td>12.33±0.99</td>
<td>62.50±0.89</td>
<td>24.53±1.0</td>
<td>15.58±1.03</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>16.25±1.16</td>
<td>51.81±1.43</td>
<td>15.17±0.97</td>
<td>68.76±0.95</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean of triplicates±standard deviation. Data were analyzed by ANOVA one way followed with Post Hoc for multiple comparisons. Groups have the same letter had no significant difference between them.
Scrophularia hypericifolia showed the best result as superoxide radical scavenger, Table (2). It scavenged phenazine methosulphate (PMS) in phenazine methosulphate-nicotinamide adenine di nucleotide (NADH) systems with the highest inhibition percentage than the other plants. It had \( IC_{50} = 20.83 \mu g/ ml \) which was less than half of standard reference \( IC_{50} \) followed with Rhazya stricta \( IC_{50} = 37.51 \mu g/ ml \) while the lowest scavenger was Emex spinosa \( IC_{50} = 68.18 \mu g/ ml \).

Scrophularia hypericifolia also produced the highest ability to scavenge hydrogen peroxide radicals \( IC_{50} = 8.66 \mu g/ ml \) and it was better than standard reference, showed nearly half of \( IC_{50} \) of standard (Table, 2). However, Emex spinosa produced the least peroxi radical scavenging effect \( IC_{50} = 28.34 \mu g/ ml \) while Rhazya stricta had the same effect of reference compound, VC \( IC_{50} = 15 \mu g/ ml \). Citrullus colocynthis and Caylusea hexagyna produced the same effect against hydroperoxi radicals \( IC_{50} \approx 25 \mu g/ ml \).

Although Emex spinosa and Caylusea hexagyna showed the highest \( IC_{50} \) for superoxide radical and hydroperoxi radical scavenging it produced the least \( IC_{50} \) for ferrous ion chelation \( IC_{50} = 15 \mu g/ ml \), Table (2). On the other hand, the lowest \( IC_{50} \) produced by Scrophularia hypericifolia as radical scavenger showed the highest \( IC_{50} \) in ion chelation \( IC_{50} = 20.51 \mu g/ ml \) that means the lowest effect but remained better than standard reference compound Vc. Generally, all tested extracts have excellent metal chelation then vitamin C at least three times for the lowest extract.

![Figure 1](http://dx.doi.org/10.4314/ajtcam.v12i3.12)

**Figure 1**: Antioxidant properties of plant extracts at different concentrations (25, 50, 100, 200 & 400µg/ml) as well as extracts total phenol content

**Antioxidant properties of plant extracts by different assays**

The five plant extracts were tested for their reduction capability. The reduction capability was increased gradually with increasing the plant concentration, the highest concentration the highest reduction effect. The best reducing agent was Emex spinosa followed with Citrullus colocynthis while the lowest reduction effect was recorded with Rhazya stricta and Scrophularia hypericifolia, they showed nearly the same results at the same concentrations, Figure (1).

The total antioxidant capacity was determined for plant extracts at the different concentrations by testing their abilities to scavenge the ABTS radicals. The tested plant extracts scavenged ABTS radicals gradually with increasing the concentration to scavenge 100% of radicals at 400 µg/ml with Rhazya stricta and Scrophularia hypericifolia (Fig., 1). However, Emex spinosa and Caylusea hexagyna showed the lowest effect as compared to all extracts.

The effect of plant extracts as scavenger and/or reducing agent reflected on their abilities to reduce lipid peroxidation determined by linoleic acid system. The most inhibitor extract for lipid peroxidation was Emex spinosa which inhibited lipid peroxidation by 77.55% at 50 µg/ml and completely inhibited peroxidation (100% inhibition) at 200 µg/ml while Citrullus colocynthis reach the same percentage (100%) at 400 µg/ml (Fig., 1) on the other hand, Caylusea hexagyna exhibited the lowest inhibiting effect at all concentration followed with Scrophularia hypericifolia.
Citrullus colocynthis had the highest amount of total phenolic compounds (350mg/g dry matter) followed with Caylusea hexagona (345mg/g), Scrophularia hypericifolia (340mg/g), Emex spinosa (250mg/g) and Rhazya stricta (145mg/g).

Discussion

Alzheimer disease (AD) is a progressive neurodegenerative disorder with multiple etiologies, the molecular mechanism of which is not yet known. Many factors and pathways have been shown to be important to or to be involved in the pathogenesis of AD. In recent years, there has been growing interest in finding natural antioxidants, including volatile chemicals, in plants because they inhibit oxidative damage and may consequently prevent inflammatory conditions (Khanha et al., 2007), ageing and neuro degenerative disease (Fusco et al., 2007). Alzheimer’s disease (AD) is the most common form of neurodegenerative disorders, neurochemically characterised by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in the basal forebrain. Symptoms ofAD and other forms of dementia can be treated by the use of agents which restore the level of acetylcholine through inhibition of both the two major forms of cholinesterase: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In late stages of AD, levels of AChE decline by up to 85% and BChE represents the predominant cholinesterase in the brain. BChE, primarily associated with glial cells but also with specific neuronal pathways, cleaves ACh in a manner similar to AChE to terminate its physiological action. Recently, demonstrated that the inhibition of AChE holds a key role not only to enhance cholinergic transmission in the brain but also to reduce the aggregation of β-amyloid and the formation of the neurotoxic fibrils in AD (Hodges, 2006).

Data of the present work showed the moderate inhibitory effect of Citrullus colocynthis, Emex spinosa, Rhazya stricta on acetylcholinesterase. They inhibited acetylthiocholine iodide hydrolysis at all concentrations in a dose dependent manner to reach the maximum values of inhibition at 400 μg/ml. However, the other two plant extracts, Scrophularia hypericifolia and Caylusea hexagona, showed weak activity against acetylcholinesterase. Selective cholinesterase inhibitors, free of dose-limiting side effects, are not currently available, and current compounds may not allow sufficient modulation of acetylcholine levels to elicit the full therapeutic response (Felder et al., 2000). In addition, some of the synthetic medicines used e.g. tacrine, donepezil and rivastigmine have been reported to cause gastrointestinal disturbances and problems associated with bioavailability (Melzer, 1998; Schulz, 2003). Therefore, the search for new AChEIs, particularly from natural products, with higher efficacy continues.

Recent studies have pointouted that AD is associated with inflammatory processes. B-Amyloid peptides contained in the senile plaques found in AD brain can induce these inflammatory processes in which radical oxygen species (ROS) are liberated, among other components (Vina et al., 2004; Stuchbury and Munch, 2005). ROS are able to damage cellular constituents and act as secondary messenger in inflammation. Antioxidants can scavenge ROS and can also attenuate inflammation pathways. The use of antioxidants may be useful in the treatment of AD [(Calabrese et al., 2003; Gibson and Huang, 2005).

Conclusion

Emex spinosa and Citrullus colocynthis exhibited the most metal chelation and reduction capability while Scrophularia hypericifolia and Rhazya stricta were the best superoxide and hydroperoxy radicals with higher total antioxidant capacities. Citrullus colocynthis, Emex spinosa were also very effective in the inhibition of AChE, DPPH radical scavenger, reducer and as metal ion chelator. Therefore, may help in preventing or alleviating patients suffering from AD as they showed both inhibitory activity of AChE and antioxidant activity. Finally, it is interesting to note that herbs which have been used for a long time as food, condiments and medicine may have properties that may suggest new applications for them in drug industries to avoid the side effects of synthetic drugs.

Acknowledgment

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References


