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Antioxidant and anti-acetylcholinesterase activities of *Zygophyllum album*

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Abstract

The present study was carried out to explore the anti-acetylcholinesterase activity and antioxidant effect as well as content of phenolic compounds of various extracts from *Zygophyllum album*. It was observed that dichloromethane and ethanol extracts were endowed with a powerful anti-acetylcholinesterase activity with IC_{50} values of 40 and 58 µg/mL, respectively. These two extracts exhibited the highest DPPH radical scavenging activity (IC_{50}=0.2 mg/mL), the highest total antioxidant capacity (185.2 and 222.4 mg vitamin E/mg extract, respectively), the highest reducing power effect, and the highest β-carotene bleaching capacity. The findings showed that the extents of anti-acetylcholinesterase activity of *Z. album* extracts as well as their antioxidant capacity are in accordance with their phenolic contents. Hence, *Z. album* leaves would be useful against Alzheimer's disease.

Introduction

Oxidative stress, caused by reactive oxygen species (ROS), is known to result in the oxidation of biomolecules, thereby leading to cellular damage and it plays a key pathogenic role in the aging process (Su et al., 2008). In recent decades, there has been growing interest in finding natural antioxidants in plants because they inhibit oxidative damage and may consequently prevent aging and neurodegenerative diseases (Fusco et al., 2007).

Neurodegenerative diseases are now-a-days also one of the major concerns due to the increase in life expectations especially Alzheimer's disease. Therefore, the search for new acetylcholinesterase inhibitors, particularly from natural products, with higher efficacy continues. In terms of biodiversity, the flora of Tunisia represents one of the richest areas in the mediterranean and comprises a very important resource of medicinal plants.

Zygophyllum album belonging to the family *Zygophyllaceae* (Mnafgui et al., 2012) is widespread in the deserts and salt marshes of southern Tunisia. The leaf, stem and fruit of this plant are used as a drug active against rheumatism, gout, asthma, diuretic, antidiabetic, antihistaminic, antihyperlipidemic, antioxidant (Mnafgui et al., 2012; Tigrine et al., 2011; Mnafgui et al., 2014; Ghoul et al., 2012; Ksouri et al., 2013), antiseptic, antispasmodic, antieczema, antidiarrheal, and anti inflammatory agents (Viqar et al., 2006; Smati et al., 2004; Tahraoui et al., 2007).

The present study was undertaken to examine the antioxidant property and anti-acetylcholinesterase enzyme inhibitory activity of *Z. album* extracts. In addition, the total phenolic, flavonoid and proanthocyanidin contents of the extracts were determined.

Materials and Methods

Collection of plant material

Leaves of *Z. album* were collected from the local Douz - Kebeli (south of Tunisia) in July 2013. The leaves were powdered and stored in sterile containers for further

use. A voucher specimen (Number LCSN 120) has been deposited in the Herbarium Laboratory of Chemistry of Natural Products, Faculty of Sciences, Sfax University, Tunisia.

Preparation of the extracts

Air-dried and powdered leaves (800 g) of *Z. album* were macerated with 80% aqueous-methanol for 24 hours three times at room temperature using a mechanical stirrer. The extract was filtered through filter paper and concentrated with a vacuum evaporator. The obtained aqueous phase was further fractionated through solvent –solvent partitioning using successively hexane, dichloromethane, ethyl acetate and *n*-butanol. After solvent evaporation, four fractions of hexane (2.7 g), dichloromethane (3.8 g), ethyl acetate (69.0 g), and *n*-butanol (80.6 g) were obtained. These fractions were stored in darkness at 4°C.

On the other hand, three aliquots (200 g each) of powdered leaves were extracted separately by maceration using three different solvents: acetone, ethanol and methanol (500 mL each). After filtration each extraction was ran at room temperature (24 hours, three times) and evaporated to dryness under vacuum to afford acetone extract (2.1 g), ethanol extract (20.0 g) and methanol extract (36.7 g).

Phytochemical analysis

Preliminary phytochemical screening for the presence of sterols, quinones, alkaloids and flavonoids was carried out by the reported protocol (Kagithoju et al., 2013).

Test for sterols

Each extract (1 mL) was mixed with a few drops concentrated sulfuric acid. The color changed from blue to green in some samples indicated the presence of sterols.

Test for quinines

Each extract (1 mL) was mixed with 2 mL of sodium hydroxide (0.1 M). The formation of pink/violet or red color indicated the presence of quinones.

Test for flavonoids

Each extract (1 mL) was mixed with a few drops of 1% aluminum chloride solution, a persistent yellow coloration indicated the presence of flavonoids.

Test for alkaloids

Each extract (1 mL) was mixed with 0.5 mL of 1% hydrochloric acid followed by a few drops of Mayer's reagent. The presence of alkaloids was indicated by the formation of cream or yellow precipitate.

Determination of phenolics content

The total phenolics content in extract was determined with Folin-Ciocalteau reagent using the method in the literature (Chen et al., 2007). A standard curve must be first plotted using gallic acid as a standard. Different concentrations of gallic acid were prepared in methanol, and their absorbances were recorded at 750 nm. 100 µL of diluted sample was added to 2 mL of 2% Na_2CO_3 aqueous solution. After 2 min, 100 µL of 50% Folin-Ciocalteau reagent was added. The final mixture was shaken and then incubated, at room temperature in the dark, for 30 min. The absorbance of all samples was measured at 750 nm, and the results were expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract).

Determination of total proanthocyanidins content

Determination of the total proanthocyanidins was based on the procedure of Sun et al (1998). A volume of 0.5 mL of 1 mg/mL extract solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid. The mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Total proanthocyanidins content was expressed in mg catechin equivalents per gram extract (mg CE/g extract).

Acetylcholinesterase enzyme inhibitory activity

Microplate assay for inhibition of acetylcholinesterase

Inhibition of acetylcholinesterase biosynthesis by plant extracts was investigated using microplate assays. The enzyme activity was measured by observing the increase of yellow color produced from thiocholine when it reacts with the 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB).

The assay for measuring acetylcholinesterase activity was based from the assay described by Ellman et al. (1961) and Ingkaninan et al. (2000) with modifications. Briefly, 125 µL of 3 mM DTNB, 50 µL of sodium phosphate buffer (pH 8.0), 25 µL of the sample dissolved in DMSO and 25 µL of 0.5 U/mL acetylcholinesterase were added in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 25 µL of acetylthiocholine iodide. The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 405 nm.

The microplate was then read at 405 nm every 3 min by a microplate reader (Bio-Tek Instrument). Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the absorbance before adding the enzyme. Every experiment was done in triplicate.

The percentage inhibition was calculated using the following equation:

Box 1: Determination of flavonoid contents

Principle

There are formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminum chloride (maximum absorbance at 430 nm). Aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. For building the calibration curve, quarcetin is used as a standard material. Various concentrations of standard quarcetin solution were used to make a standard calibration curve.

Requirement

Test tubes, test tube rack, aluminum foil to wrap the tube, Eppendorf micropipette with microtips, electronic balance, spectrophotometer, quercetin, aluminum trichloride, methanol

Procedure

Step 1: Seven tubes were taken and wrapped with aluminum foil in order to protect extract from light

Step 2: Weigh 1 mg of each extract into the tube using an electronic balance

Step 3: Dilute each extract with 1 mL of methanol

Step 4: Add 1 mL of 2% aluminum trichloride into the methanolic solution and mixed it well

Step 5: Close the tubes with cap

Step 6: Incubate the samples at room temperature for 15 min

Step 7: Switch on the spectrophotometer. The absorbance of the reaction mixture was taken at 430 nm

Calculation

The total flavonoids content was expressed in mg quercetin equivalents per gram of extract (mg QE/g extract).

Reference

Djeridane et al., 2006.

$PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound

Extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting the percentage inhibition against extract concentration. Tacrine was used as positive control.

Chemicals

Acetylthiocholine iodide, acetylcholinesterase type V-S, from electric eel, DTNB and tacrine were obtained from Sigma. Sodium phosphate pH 8.0 was used as a buffer throughout the experiment unless otherwise stated. The enzyme stock solution was kept at (-80°C). The further enzyme-dilution was dissolved in buffer. DTNB and acetylthiocholine iodide were dissolved in the buffer.

Antioxidant activities

Ferric-reducing antioxidant power assay

The method of Yildirim et al. (2001) was used to assess the reducing power of all *Z. album* extracts. Briefly, extracts (1 mg) were dissolved in 1 mL of distilled water and mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance of the mixture was measured at 700 nm. The increased absorbance of the mixture indicated increased reducing power. A standard curve was prepared using various concentrations of ascorbic acid.

Total antioxidant capacity

The antioxidant activity of all extracts of *Z. album* was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex. An aliquot of (0.1 mL) of each extract (1 mg/mL) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).

The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer UV–Vis against blank after cooling to room temperature. Methanol (0.3 mL) in place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of vitamin E.

Determination of DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding extracts was measured from the bleaching of the purple-colored methanol solution of DPPH.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging effect was evaluated following the procedure described in a previous study (Tepe et al., 2006). Fifty microliters of various concentrations of the extracts dissolved in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical,

Table I

Phytochemical screening of *Zygophyllum album* extracts

Test	*n*-Hexane	Dichloromethane	Ethyl acetate	Acetone	Butanol	Ethanol	Methanol
Sterols	+	-	-	-	-	-	-
Quinones	-	+	+	-	+	+	-
Flavonoids	-	+	+	+	+	+++	+
Alkaloids	-	+	-	-	-	+	+

(+): presence ; (-) : absence

DPPH, in percent (PI%) was calculated in following way:

$PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. The synthetic antioxidant reagents butylate hydroxytoluene (BHT) and vitamin E were used as positive control and all tests were carried out in triplicate.

β-carotene bleaching method

The ability of *Z. album* extracts to prevent bleaching of β-carotene was assessed as described by Koleva et al. (2002). A stock solution of β-carotene/linoleic acid mixture was prepared by dissolving 0.5 mg of β-carotene, 25 µL of linoleic acid and 200 µL of Tween 40 in 1 mL of chloroform. The chloroform was completely evaporated under vacuum in a rotatory evaporator at 40°C, then 100 mL of bidistilled water were added, and the resulting mixture was vigorously stirred. The emulsion obtained was freshly prepared before each experiment. Aliquots 2.5 mL of the β-carotene/linoleic acid emulsion were transferred to test tubes containing 0.5 mL of each extracts (0.6 mg/mL). Following incubation for 2 hours at 50°C, the absorbance of each sample was measured at 470 nm. Measurement of absorbance was continued until the color of the β-carotene disappeared in the control tubes (t=120 min) at intervals of 15 min. BHA was used as positive standard. A blank consisted of 0.5 mL of bidistilled water instead of sample.

The antioxidant activity (AA%) of the *Z. album* extracts was evaluated in terms of bleaching of β-carotene using the following formula:

$AA\% = [1 - ((A_0 - A_t) / (A'_0 - A'_t))] \times 100$

A_0 and A'_0 are the absorbances of the sample and the blank, respectively, measured at time zero. A_t and A'_t are the absorbances of the sample and the control, respectively, measured after incubation for 120 min. The same procedure was repeated with BHA as positive control.

Statistical analysis

The data was analyzed using the statistical software program SPSS 18.0 for Windows. All results were given as mean ± standard division (SD). The potential correlation among the acetylcholinesterase inhibitory, antioxidant capacity, total phenol, flavonoid and pro-anthocyanidin contents were analyzed and differences at $p<0.05$ were considered as significant, $p<0.01$ highly significant and $p>0.05$ non-significant.

Results

The extraction yields obtained with methanol (18.3%), butanol (10.0%), ethanol (10.0%) and ethyl acetate (8.6%) were higher than those obtained with acetone (1.0%), dichloromethane (0.4%) and *n*-hexane (0.3%). This was due to the affinity between polarity of solvents and extracted compounds.

Only *n*-hexane extract tested positive for the presence of sterols, while dichloromethane, ethanol and methanol extracts were positive tested for quinones, flavonoids and alkaloids (Table I). The other extracts showed positive test of quinones and flavonoids.

Based on the absorbance values of the extracts solutions compared with the standard solutions of gallic acid, quercetin and catechin, the total phenolic, flavonoid and proanthocyanidin contents are shown in Figure 1. Except the *n*-hexane extract, which contained low amounts of phenolic compounds, the other extracts were found to be rich in these compounds.

As shown in Figure 1, the butanol extract showed the highest amount of phenolic compounds (403.4 mg GAE/g extract) followed by ethyl acetate (394.5 mg GAE/g extract), dichloromethane (392.5 mg GAE/g extract), ethanol (391.6 mg GAE/g extract), methanol (219.7 mg GAE/g extract) and acetone (218.2 mg GAE/g extract) extracts.

Among all extracts, the highest amount of flavonoids was found in methanol extract (120.2 mg QE/g extract). However, the acetone extract showed the highest amount of proanthocyanidins (190.9 mg CE/g extract).

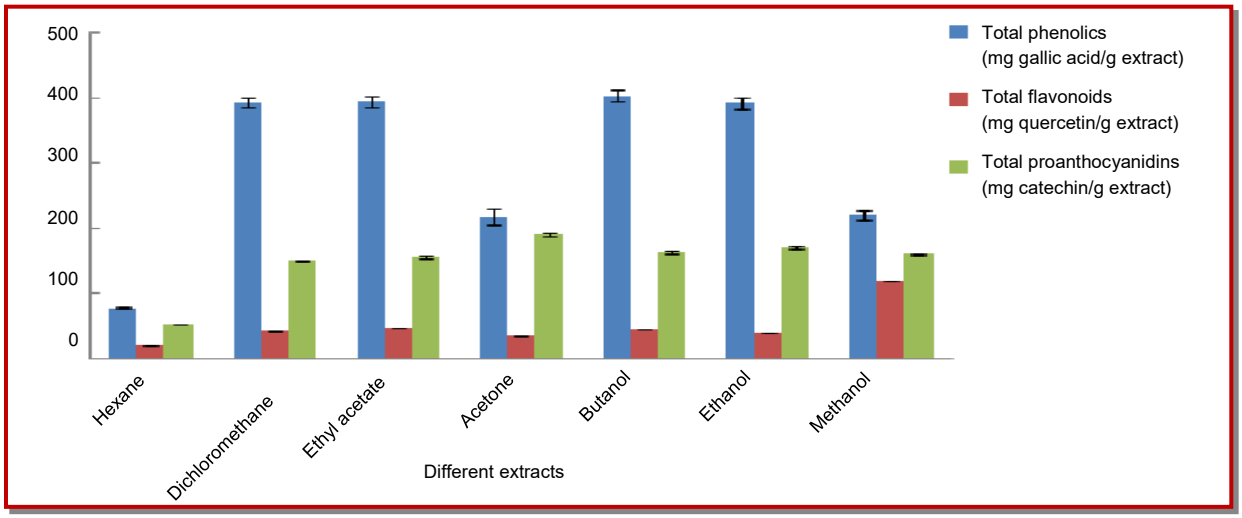

Figure 1: Total phenolic, flavonoid and proanthocyanidin contents of *Zygophyllum album* extracts

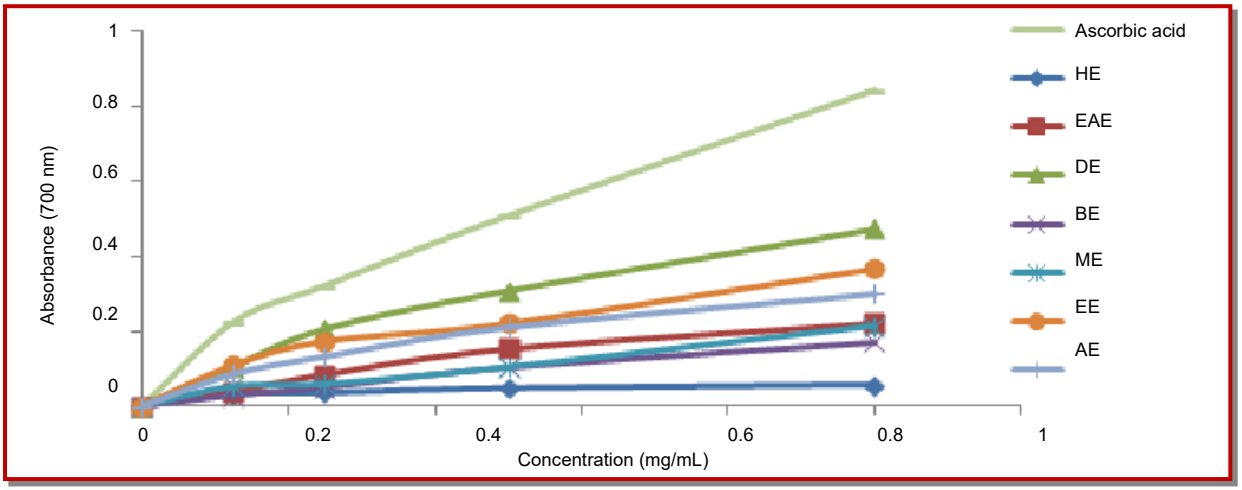

Figure 2: Reducing power activity of *Zygophyllum album* extracts

The acetylcholinesterase enzyme inhibition of the extracts was determined using Ellman's colorimetric method in 96-welled microplate. At the concentration of (1 mg/mL), only dichloromethane and ethanol extracts gave a strong acetylcholinesterase inhibition with IC_{50} values of 40 µg/mL and 58 µg/mL, respectively (Table II). Tacrine was used as a standard of acetylcholinesterase inhibition with an IC_{50} value of 35 µg/mL. The methanol (IC_{50}=120 µg/mL) and ethyl acetate (IC_{50}=400 µg/mL) extracts showed moderate acetylcholinesterase inhibition activity. However, the other extracts were not active.

Figure 2 showed that ascorbic acid (positive control) had significantly higher antioxidant activity. Furthermore, the *Z. album* leaves extracts exhibited a reducing power activity which decreased in this order:

Dichloromethane > ethanol > acetone > ethyl acetate > butanol > methanol > *n*-hexane.

This result suggested that the tested extracts have a good ability to donate electrons to reactive free radicals, converting them into more stable products.

Table II

Acetylcholinesterase inhibitory activity of *Zygophyllum album* extracts

Extracts	IC_{50} (µg/mL)
n-Hexane	Not detected
Dichloromethane	40.0 ± 3.3
Ethyl acetate	400.0 ± 6.6
Acetone	Not detected
Butanol	Not detected
Ethanol	58.0 ± 0.6
Methanol	120.0 ± 6.6
Tacrine	35.0 ± 3.3

The total antioxidant capacity of various extracts from *Z. album* leaves is given in Figure 3 and it was expressed as the number of equivalents of vitamin E. High absorbance values indicate that the sample

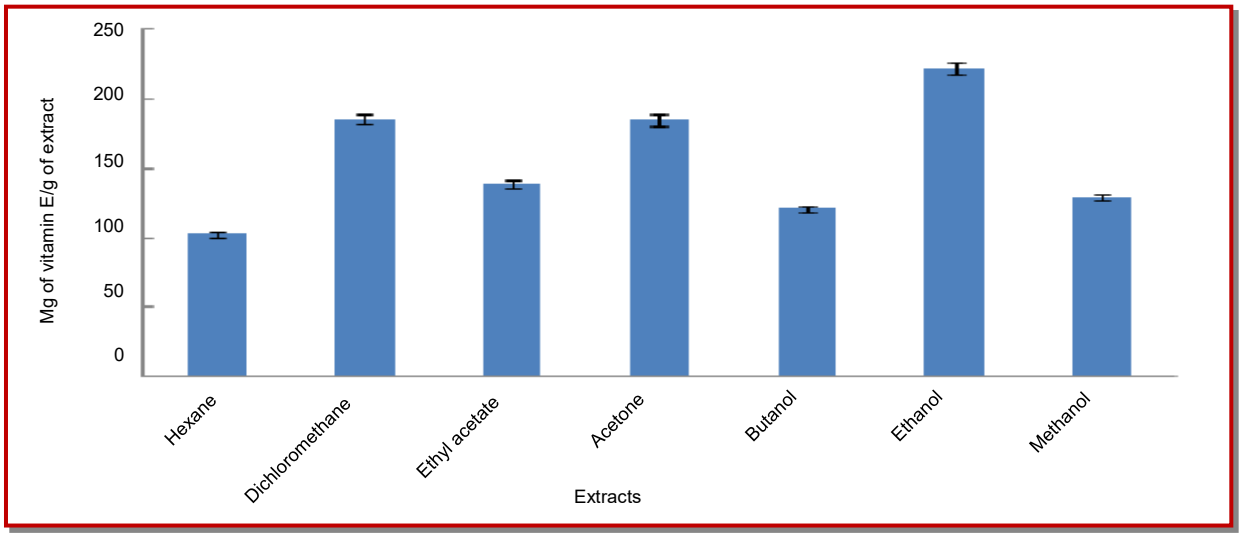

Figure 3: Total anti-oxidant capacity of *Zygophyllum album* extracts

Table III

Anti-oxidant activity of of some extracts from *Zygophyllum album*. L

Extracts	IC_{50} (µg/mL)
n-Hexane	0.4 ± 0.1
Dichloromethane	0.2 ± 0.1
Ethyl acetate	0.3 ± 0.1
Acetone	0.3 ± 0.1
Butanol	0.3 ± 0.1
Ethanol	0.2 ± 0.1
Methanol	0.3 ± 0.1
BHT	0.01 ± 0.3
Vitamin E	0.02 ± 0.5

possesses significant antioxidant activity. The anti-oxidant capacity of the different extracts was found to decrease in this order:

Ethanol > dichloromethane > acetone > ethyl acetate > butanol > methanol > *n*-hexane.

The free radical scavenging capacity was analyzed using DPPH method. DPPH scavenging activities of *Z. album* extracts and standard antioxidants are given in Table III. IC_{50} values of extracts stood between 0.2 and 0.4 mg/mL. The highest antioxidant activity (IC_{50} = 0.2 mg/mL) was related to the dichloromethane extract. The order of DPPH scavenging activity of extracts was:

Dichloromethane > ethanol > acetone > ethyl acetate > methanol > butanol > *n*-hexane.

Although the IC_{50} of various extracts were significantly lower than those of BHT and vitamin E, it was evident that the extracts show the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

In the β-carotene bleaching system, β-carotene undergoes rapid discoloration in the absence of antioxidants. The addition of the tested extracts to this system prevents the bleaching of β-carotene at different degrees. Figure 4 showed that the percentage of β-carotene bleaching inhibition by *Z. album* extracts decreased with the time. The absorbance of *n*-hexane extract decreased rapidly, indicating that the anti-oxidant activity of this extract was low, whereas the dichloromethane extract hindered the extent of β-carotene bleaching on a dose-dependent manner (data note shown).

Discussion

Phenolic compounds (phenols, flavonoids and pro-anthocyanidins) received considerable attention because of their potential antioxidant activity (Suhartonoa et al., 2012; Pavithra and Vadivukkarasi, 2015; Khlif et al., 2015; John and Shahidi, 2010). These compounds are known for their ability of scavenging free radicals and active oxygen species such as single oxygen (Lahlou et al., 2014). According to our findings, the total phenolic contents of extracts ranged from 78.0 ± 1.5 to 403.4 ± 8.0 mg gallic acid/g extract. Total flavonoids, expressed as quercetin equivalents, varied from 21.8 ± 0.1 to 120.2 ± 0.2 mg/g extract. However, total proanthocyanidin contents in extracts found to be in a range from 52.9 ± 0.5 to 190.9 ± 2.3 mg/g extract, as catechin equivalents. These results showed that *Z. album* extracts may be suggested as a potential source of natural phenolic compounds which are endowed with several activities especially antioxidant activity (Keskes et al., 2014). On the other hand, plants have been used traditionally to enhance cognitive function and to alleviate other symptoms associated nowadays with Alzheimer's disease (Natarajan et al., 2013). Acetylcholinesterase inhibition is an important drug treatment

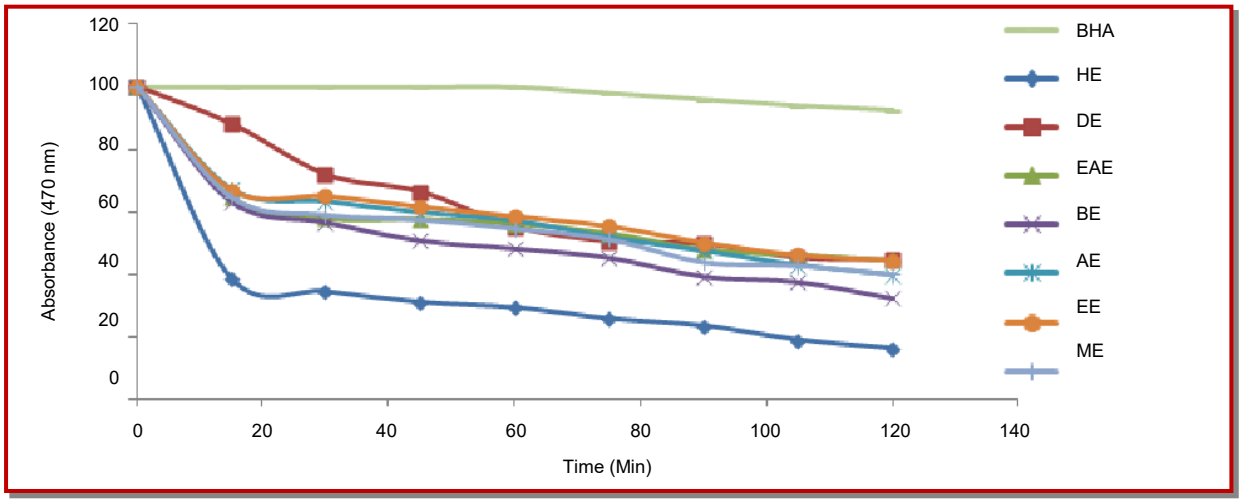

Figure 4: β-Carotene bleaching assay of *Zygophyllum album* extracts

strategy against Alzheimer's disease and, recently, there has been considerable interest in finding naturally acetylcholinesterase inhibitors to replace synthetic drugs such tacrine and donepzil (having some adverse effects) (chatiiparkon et al., 2007). For this purpose, our data revealed that *Z. album* could serve as inhibitor against cholinesterase enzyme family. Our results revealed that the dichloromethane and ethanol extracts gave a strong acetylcholinesterase inhibition with IC_{50} values of 40 µg/mL and 58 µg/mL, respectively when compared to that of tacrine (IC_{50} of 35 µg /mL). The methanol and ethyl acetate extracts showed moderate acetylcholinesterase inhibition (120 µg/mL and 400 µg/mL, respectively). The inhibitory action of *Z. album* extracts against acetylcholinesterase activity would be related to their content of polyphenols, flavonoids, sterols and alkaloids. Recently, it was reported that these compounds have been shown to possess anti-acetylcholinesterase properties (Ji and Zhang, 2008; Farlow, 2009; Sacan and Yanardag, 2010; Zhao et al., 2013; Moyo et al., 2010; Cao et al., 2013).

In the present study, the ferric-reducing power, the total antioxidant capacity, the DPPH radical-scavenging and the β-carotene bleaching systems were used to evaluate the antioxidant activity of *Z. album* extracts.

The reducing power of an extract is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power activity of test specimen. Therefore, in this study, reducing activity was determined based on the ability of extracts to reduce a Fe^{3+} ferricyanide complex to form a Fe^{2+} ferrous complex. The amount of Fe^{2+} was monitored by measuring the formation of perl's prussian blue at 700 nm (Ebrahimzadeh et al., 2010).

The reducing power of the different extracts was found to decrease in this order:

Dichloromethane > ethanol > acetone > ethyl acetate > butanol > methanol > n-hexane.

The data suggest that *Z. album* extracts have a good ability to donate electrons to reactive free radicals, converting them into more stable products. This result can be related to phenolic compounds which were quantified in the extracts. Those compounds have a good ability to donate electrons to reactive free radicals, converting them into more stable products and terminating the free radical chain reaction (Sun et al., 2011).

The total antioxidant activity was analyzed by phosphomolybdenum method based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm (Prasad et al., 2009; Zhao et al., 2008). Electron transfer occurring in this assay depends upon the structure of the antioxidant (Kammoun et al., 2011).

The antioxidant capacity of the different extracts was found to decrease in this order:

Ethanol > dichloromethane > acetone > ethyl acetate > butanol > methanol > hexane.

This result is in full agreement with that obtained from the Ferric-reducing antioxidant power assay.

On the other hand, the free radical scavenging activity of *Z. album* extracts was evaluated using the DPPH test. This test aims to measure the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picryl hydrazil (DPPH) formed in solution by donation of a hydrogen atom or an electron (Tepe et al., 2006). DPPH gives a strong absorption band at 517 nm in visible spectroscopy. The stable free radical DPPH has been widely used to test the free radical-scavenging capacity of various antioxidants (Pavithra and Vadivukkarasi, 2015). If the extracts have the capacity to scavenge the DPPH free radical, the initial purple solution will

change to a yellow color due to the formation of diphenylpicrylhdrazine.

Dichloromethane, acetone and ethanolic extracts showed the highest antioxidant activities compared to those of BHT and vitamin E (standard antioxidants).

In our study, the inhibitory effect on lipid peroxidation of *Z. album* extracts was determined by β-carotene/ linoleic acid bleaching test. The mechanism of β-carotene bleaching is a free radical–mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. β-Carotene in this model undergoes rapid discoloration in the absence of antioxidants. The linoleic acid free radical, formed on abstraction of a hydrogen atom from its diallylic methylene group, attacks the highly unsaturated β-carotene molecules. As β-carotene loses its double bonds by oxidation, the compound loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically (Yang et al., 2008).

The presence of extract with antioxidant activity can hinder the extent of β-carotène bleaching by neutralizing the linoleate-free radical formed in the system (Barros et al., 2007). The reduction in absorbance of β-carotene-linoleate emulsion in presence of the extracts was shown in Figure 4.

Among the tested extracts, dichloromethane, ethanol and acetone extracts were endowed with the powerful inhibition capacity against linoleic acid, while hexane extract exhibited the lowest antioxidant activity.

Conclusion

The crude extracts from *Z. album* exhibited a powerful antioxidant activity, which can be ascribed to their different phenolic compositions. Furthermore, the antiacetylcholinesterase assay showed that dichloromethane and ethanol extracts of *Z. album* are endowed with potent activity, indicating that these two extracts can be used in the prevention of neurodegenerative diseases such as Alzheimer's disease.

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Conflict of Interest

Authors declare no conflict of interest

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