

Chemical Composition and Biological Activities of *Zygophyllum album* (L.) Essential Oil from Tunisia

M. Kchaou¹, H. Ben Salah^{1*}, K. Mnafgui², R. Abdennabi³, N. Gharsallah³, A. Elfeki², M. Damak¹, and N. Allouche¹

ABSTRACT

The aim of the present study was to investigate the antioxidant, anti-diabetic, anti-obesity and anti-bacterial activities and the chemical composition of essential oil of *Zygophyllum album* leaves (*Zygophyllaceae*) harvested from Douz (Tunisia). GC-MS analysis of essential oil of *Z. album* resulted in the identification of nineteen compounds representing 91.55% of the total oil. 2,6-Di(tert-butyl)phenol, delta decalactone, cocolactone and carvacrol were the principal components comprising 63.65% of the oil. The antioxidant activity of essential oil of *Z. album* was evaluated using 2,2-DiPhenyl-1-PicrylHydrazyl (DPPH), reducing power and total antioxidant capacity assays. The obtained results reveal that the essential oil of *Z. album* is endowed with potent antioxidant property. In addition, the IC_{50} value of essential oil of *Z. album* against α -amylase was $43.17 \mu\text{g mL}^{-1}$ and that against pancreatic lipase was $85.95 \mu\text{g mL}^{-1}$, suggesting powerful anti-diabetic and anti-obesity effects, respectively. Moreover, the essential oil of *Z. album* showed a significant anti-bacterial activity especially against Gram (+) bacteria. This study suggests that essential oil of *Z. album* may be used as a potential natural source for anti-oxidative, anti-diabetic and anti-microbial agents in drug and food industries.

Keywords: Anti-bacterial activity, Anti-diabetic, Antioxidant property, Anti-obesity activity, Pancreatic lipase inhibition.

INTRODUCTION

Zygophyllum album L. is a shrubby plant, belonging to *Zygophyllaceae* family which includes about 27 genera and 285 species frequently restricted to arid and semiarid areas. (Mnafgui *et al.*, 2012; Beier *et al.*, 2003).

Zygophyllum species are widely used in traditional medicine for their anti-diabetic (Eskander and Won Jun, 1995; Jaouhari *et al.*, 2000), anti-spasmodic, anti-eczemic (Sasmakov *et al.*, 2001), anti-septic, anti-diarrhoeal (Atta and Mouneir, 2004, Meng *et*

al., 2002) and anti-inflammatory effects (Rimbau *et al.*, 1999; Ksouri-Megdiche *et al.*, 2013). *Zygophyllum album* is one of several traditional remedies used as diuretic, anti-diabetic (Mnafgui *et al.*, 2014, Tigrine-Kordjani *et al.*, 2011), anti-histaminic, anti-hyperlipidemic, antioxidant (Ghoul *et al.*, 2012; Ksouri-Megdiche *et al.*, 2013); anti-septic and carminative agents (Ksouri-Megdiche *et al.*, 2013). It is also used against rheumatism, gout, asthma, hypertension and treatment of dental caries (Tigrine-Kordjani *et al.*, 2011).

¹ Laboratory of Chemistry of Natural Substances, University of Sfax, Faculty of Sciences of Sfax, P. B. "1171", 3000, Sfax, Tunisia.

* Corresponding author; email: ben_salah9@yahoo.fr

² Laboratory of Animal Ecophysiology, University of Sfax, Faculty of Sciences of Sfax, P. B. "1171", 3000, Sfax, Tunisia.

³ Laboratory of Plant Biotechnology Applied to Crop Improvement, Faculty of Sciences of Sfax, P. B. "1171", 3000, Sfax, Tunisia.



Essential oils have been widely used throughout history for their pharmacological activities such as antioxidant, anti-diabetic, analgesic, anti-inflammatory, spasmolytic, antiseptic, antibacterial, antifungal, antiviral (Bakkali *et al.*, 2008; Burt, 2004; Batish *et al.*, 2008), anti-parasitic and antispasmodic properties (Teuscher, 2006).

More than 250 types of essential oils are traded in the world market and a big number of countries produce different kinds of essential oils (Rajeswara Rao *et al.*, 2005). This has recently attracted the attention of many scientists and encouraged them to study the biological activities of their compounds from chemical and pharmacological investigations to therapeutic aspects (Ceccarini *et al.*, 2004).

The chemical composition of *Zygophyllum album* essential oil has been previously studied (Tigrine-Kordjani *et al.*, 2011), but, to the best of our knowledge, there are no published reports on its biological activities. This study was conducted to investigate for the first time antioxidant, antibacterial, anti-diabetic and anti-obesity activities of *Zygophyllum album* essential oil grown in Tunisia.

MATERIALS AND METHODS

Plant Material

Zygophyllum album was collected from region of Douz (Kebili, Region 6, south of Tunisia) in September 2012. The taxonomic identification of the plant material was confirmed by Professor Mohamed Chaieb in the botany laboratory of the Faculty of Sciences, Sfax University, Tunisia. A voucher specimen (Number LCSN 120) has been deposited in the Herbarium Laboratory of Chemistry of Natural Products, Faculty of Sciences, Sfax University, Tunisia.

Extraction of Essential Oil

Essential oil was obtained from freshly harvested leaves (800 g) of *Z. album* by

hydro-distillation for 3 hours in a Clevenger apparatus. When the condensed material cooled down, the water and essential oils were separated. The oil was decanted to be used as essential oil. To improve its recovery, the essential oil was taken up in diethyl ether, dried over anhydrous sodium sulfate until the last traces of water were removed and stored in a dark glass bottle at 4 °C until tested and analyzed. The yield of the obtained oil was 0.044% (w/w).

GC-MS Analysis

The analysis of the essential oil of *Z. album* was performed on a GC-MS HP. It was carried out in a Hewlett-Packard 6890-5973 system operating on EI mode, equipped with a capillary column HP-5 MS (DB-5) 30 m×0.25 mm; film thickness, 0.25 µm; temperature program, 60 (5 minutes) to 280°C at a rate of 3 °C min⁻¹; injection temperature 200°C. GC-MS analysis was also performed on a Finnigan GCQ Plus ion-trap mass spectrometer with an external ion source in both the Electronic Impact (EI) and Chemical Ionization (CI) modes at a flow rate of 1.0 mL min⁻¹ using CH₄ as the CI ionization reagent. *N*-Alkanes were used as reference points in the calculation of the Kovats Indices (KI). Tentative identification of the compounds was based on comparison of their relative retention time's literature values (Adams, 2004).

Determination of DPPH Radical Scavenging Activity

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 essential oil in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517

nm. Inhibition of free radical, DPPH, in percent (PI%) was calculated in the following way:

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

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.

Determination of Total Antioxidant Capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto, Pineda and Aguilar (Prieto *et al.*, 1999). The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. An aliquot of 0.3 mL of essential oil at different concentrations was combined with 3 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 minutes. 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 the place of extract was used as the blank. The antioxidant activity was expressed as the number of equivalents of vitamin E.

Determination of Reducing Power

The method of Yildirim *et al.* (2000, 2001) was used to assess the reducing power of essential oil of *Zygophyllum album*. An aliquot of 1 mL of different concentrations

of essential oils (5, 10, 25, 50, 100 $\mu\text{g mL}^{-1}$) was mixed with 2.5 mL of a 0.2M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$], and incubated in a water bath at 50°C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid were added to the mixture that was centrifuged at 2,000 rpm for 10 minutes. 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. Increased absorbance of the mixture indicated increased reducing power. A standard curve was prepared using various concentrations of vitamin E.

Determination of α -Amylase Inhibition Activity

The *in vitro* α -amylase inhibition activity for essential oil was determined based on the spectrophotometric assay using acarbose as the reference compound (Gella *et al.*, 1997). Sample was dissolved in DiMethyl Sulfoxide (DMSO) to give concentrations of 50, 100, and 200 $\mu\text{g mL}^{-1}$. The enzyme α -amylase solution was prepared by mixing 3.246 mg of α -amylase (EC 3.2.1.1) in 100 mL of 40 mM phosphate buffer, pH 6.9. Add 60 μL of 40 mM phosphate buffer (pH 6.9)/acarbose/sample and 30 μL of α -amylase enzyme and are preincubated at 37°C for 10 minutes, and then 120 μL of E-PNPG7 was added, mixed and incubated at 37°C for 8 minutes. The absorbance was measured at 405 nm and the control reaction was carried out without the oil. Percentage Inhibition was calculated by the following expression:

$$PI = [(Absorbance_{\text{Control}} - Absorbance_{\text{Test}}) / Absorbance_{\text{Control}}] \times 100$$

Determination of Pancreatic Lipase Inhibition Activity

The method was modified from the assay reported by Nakai *et al.* (2005), in which 4-



MethylUmbelliferyl oleate (4-MU oleate) was used as a substrate to measure the pancreatic lipase of all samples. Briefly, the assay was conducted by mixing 50 μL of the pancreatic lipase solution (2 unit mL^{-1}) in a buffer consisting of 50 mmol L^{-1} Tris HCl (pH 8.0), 100 μL of diluted sample solutions and 50 μL of 0.5 mmol L^{-1} 4-MU solution dissolved in the above buffer in the well of a 96 micro well plate to start the enzyme reaction. The plate was immediately placed in the 37°C pre-heating FLx800 micro plate fluorescence reader (Bio-Tek® Instruments, Inc., Winooski, VT) to measure the amount of 4-methylumbelliferone released by lipase every minute for 30 minutes at an excitation wavelength of 360 nm with a tolerance of ± 40 nm and an emission wavelength of 455 nm with a tolerance of ± 20 nm. The lipase inhibitive activity was determined by measuring the effect on the enzyme reaction rate after adding the extracts, compared with the control. Fluvastatin was used as positive control.

$$\text{PI} = \left[\frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \right] \times 100$$

Determination of Antibacterial Activity

The bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37°C for 18 to 24 hours prior to seeding onto the nutrient agar. One or several colonies of the respective bacteria were transferred into API suspension medium (bioMerieux) and adjusted to 0.5 McFarland turbidity standards with a Densimat (bioMerieux) (Saïdana *et al.*, 2008; Hichri *et al.*, 2003). The inocula of the respective bacteria were streaked into MH agar plates using a sterile swab and were then dried at 37°C during 15 minutes. A sterile filter disc having 6 mm of diameter was placed at the surface of MH agar and 5 μL of the essential oil was dropped onto each Whatman paper disc (Bel Haj Khetter *et al.*, 2008). The treated Petri dishes were incubated at 37°C for 18 to 24 hours. The antibacterial activity was evaluated by measuring the clear zone

surrounding the Whatman paper. Standard discs of the antibiotic ampicillin (BIO-RAD) were applied as a positive antibacterial controls.

Statistical Analyses

All analyses were performed in triplicate and the data were reported as means \pm Standard Deviation (SD). Differences between experiments were analyzed using Student's t-test in Microsoft Excel 2000 (Microsoft Corporation, USA). The confidence limits used in this study were based on 95% ($P < 0.05$).

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil of *Zygophyllum album* L.

Hydro-distillation of fresh leaves of *Z. album* gave a pale yellow oil yield of 0.044%. The chemical composition of this oil is listed in Table 1.

GC-MS analysis of the essential oil of *Z. album* led to the identification of nineteen compounds accounting for 91.55% of the total oil. The oil composition was dominated by alcohols (42%) followed by esters (36.59%). 2,6-Di(tert-butyl) phenol (18.81%), delta Decalactone (18.11%), cocolactone (13.70%) and carvacrol (13.03%) were the principal components comprising 63.65% of the essential oil of *Z. album*.

The chemical composition of essential oil of *Z. album album* was partly in agreement with that of the Algerian *Zygophyllum album* plant (Tigrine-Kordjani *et al.*, 2006, 2011). According to Tigrine-Kordjani *et al.* (2011), damascenone, delta decalactone, alpha (E) Ionone and non-3-en-2-one were the major components of the Algerian essential oil of *Z. album*. This difference in chemical composition may be due to local climatic conditions and period of plant harvest (Keskes *et al.*, 2014).

Table 1. Chemical composition of the essential oil of *Zygophyllum album* leaves.

No.	Compound	% Peak area	KI ^a	Identification ^b
1	Hyacinthine	0.78	1005	KI, MS
2	1-Nonen-4-ol	2.49	1012	KI, MS
3	Nonanal	1.71	1025	KI, MS
4	Safranal	1.19	1063	KI, MS
5	4-Vinylphenol	1.18	1071	KI, MS
6	Carvacrol	13.03	1078	KI, MS
7	Aminocaproic acid	1.60	1085	KI, MS
8	Delta octalactone	2.42	1094	KI, MS
9	Thymol	2.23	1100	KI, MS
10	Varamol	1.66	1106	KI, MS
11	Eugenol	2.60	1127	KI, MS
12	Damascenone	3.99	1140	KI, MS
13	1,2-Dihydro-1,4,6-trimethyl naphthalene	1.95	1153	KI, MS
14	n.i. ^c	1.72	1164	
15	n.i.	1.15	1177	
16	Cocolactone	13.70	1188	KI, MS
17	Delta decalactone	18.11	1197	KI, MS
18	2,6-Di(tert-butyl) phenol	18.81	1199	KI, MS
19	n.i.	1.38	1228	
20	6,10,14-trimethyl pentadecan-2-one	0.62	1335	KI, MS
21	n.i.	1.43	1379	
22	Bis(2-ethyl hexyl) phthalate	2.36	1570	KI, MS
23	2-Methyl indol	1.12	1612	KI, MS
Total		97.23		
Identification components (%)		91.55		

^a Kovats Index on HP-5MS capillary column in reference to n-alkanes. ^b Comparison of mass spectra with MS libraries, KI: Comparison of Kovats Index with bibliography. ^c NI: Non-Identified.

value of 400 $\mu\text{g mL}^{-1}$ when compared to BHT ($\text{IC}_{50} = 17 \mu\text{g mL}^{-1}$) and vitamin E ($\text{IC}_{50} = 26 \mu\text{g mL}^{-1}$).

DPPH Free Radical Scavenging Activity

Table 2 shows the scavenging effect of essential oil of *Z. album* on DPPH radical. The capability of substances to donate hydrogen to convert DPPH into the non-radical form of DPPH can be followed spectrophotometrically. Butylate HydroxyToluene (BHT) and vitamin E were used as standards. As shown in Figure 1, the essential oil of *Z. album* exhibits moderated free radical-scavenging activity with an IC_{50}

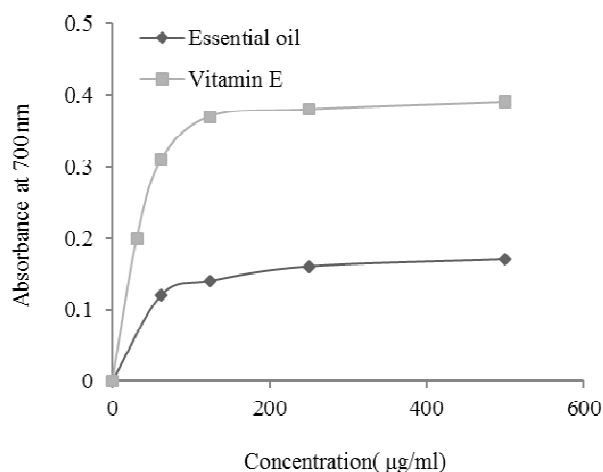
Total Antioxidant Capacity Assay

The Total Antioxidant Capacity (TAC) was evaluated by the phosphomolybdate method (Prieto *et al.*, 1999). This assay is a quantitative method to evaluate water soluble and fat-soluble antioxidant capacity (Salmanian *et al.*, 2014).

In the presence of essential oil, Mo (VI) is reduced to Mo (V) and forms a green

**Table 2.** Antioxidant activities of essential oil of *Zygophyllum album*.

Sample	Scavenging of DPPH ($\mu\text{g mL}^{-1}$)	TAC (Equivalent to vitamin E)
Essential Oil	400 \pm 2.00	382.25 \pm 7.50
BHT	17 \pm 0.08	
Vitamin E	26 \pm 0.13	

**Figure 1.** Reducing power of EOZA.

colored phosphomolybdenum (V) complex at acid pH, which shows a maximum absorbance at 695 nm. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity. Table 2 shows the total antioxidant capacity of essential oil of *Z. album*. The results indicate a concentration dependent total antioxidant capacity. It means that essential oil contains as much quantity of antioxidant compounds as equivalents of vitamin E to effectively reduce the oxidant in the reaction matrix. Antioxidant capacity of vitamin E has been used as a reference for comparison of essential oil of *Z. album* activity (Aderogba *et al.*, 2005).

Reducing Power Assay (RP)

The reducing power of a compound is related to its electron transfer ability and may, therefore, serve as a significant

indicator of its potential antioxidant activity. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Duh *et al.*, 1999; Yildirim *et al.*, 2000; Dorman *et al.*, 2003). Antioxidant potential of essential oil of *Z. album* was estimated using potassium ferric cyanide reduction method. The presence of antioxidants in the samples causes the reduction of Fe^{3+} /Ferric cyanide complex to ferrous form. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1 shows that the reducing power increases with the concentration of essential oil. In the present study, it can be concluded that the antioxidant activity of the essential oil can come from its phenolic components such as 2,6-Di(tert-butyl) phenol, carvacrol, thymol and eugenol. However, the antioxidant activity of phenolic components in the essential oil was supported by many studies (Özkan *et al.*, 2011; Mastelic *et al.*,

2008; Kaya *et al.*, 2010; Dorman *et al.*, 2000; Ruberto *et al.*, 2000).

Antibacterial Activity

Antibacterial activity of essential oil of *Z. album* was evaluated against a set of significant bacterial strains, including both Gram-positive and Gram-negative. The inhibitory effect on bacterial growth was determined using agar disc diffusion assay (Pérez *et al.*, 1999; Bagamboula *et al.*, 2002). The obtained results from this test are summarized in Table 3.

The Essential oil of *Zygophyllum album* shows a moderate antibacterial activity especially against gram-positive bacteria. Indeed, *Enterococcus faecalis*, a highly pathogenic strain, has an important sensitivity to the essential oil. The highest resistance in Gram (-) bacteria might be explained by the presence of their outer membrane covering the cell wall, which limits the diffusion of hydrophobic compounds through its lipopolysaccharide coating. The absence of this barrier at the Gram (+) allows the direct contact of the essential oils and hydrophobic components with their phospholipid bilayers of the cell membrane, which causes an increase of ion permeability and leakage of intracellular

components vital, or the reduction of the bacterial enzyme systems. Most studies have reported that plant extracts generally are more active against the Gram-positive bacteria than the Gram-negative bacteria (Gutiérrez- Larraínzar *et al.*, 2012; Salmanian *et al.*, 2014). These reports are in agreement with the results of the present study.

This potent activity may be related to the presence of carvacrol (13.03%), eugenol (2.60%) and thymol (2.23%) in the essential oil. These phenolic compounds are known for their antibacterial activity (Kavitha and Richard, 2010). The main mechanism reported for antimicrobial activity of plant extracts has been membrane disruption by phenolics. In the case of phenolic compounds cell wall lysis, cytoplasmic and bacterial protein membrane damage may cause leakage of cellular ultrastructure and, as a result, cell death (Negi, 2012).

On the other hand, it can be noted that the essential oil is inactive against strain *Klebsiella pneumoniae* WHO24. This resistance is related to the nature of the outer membrane, which confers resistance to most biocides.

Alpha-Amylase Inhibitory Assay

Alpha-amylase is an enzyme that hydrolyzes starch molecules to give diverse

Table 3. Anti-bacterial activity of essential oil of *Zygophyllum album* using agar disc diffusion.^a

Strains	DD ^a	DD ^b
Bacterial strains Gram (+)		
<i>Staphylococcus aureus</i> ATCC 6538	10.0 ± 0.4	20 ± 0.5
<i>Enterococcus faecalis</i> ^d	18.0 ± 1.0	25 ± 1.0
<i>Bacillus cereus</i> ^d	12.0 ± 0.5	21.0 ± 1.0
<i>Bacillus subtilis</i> ^d JN 934389	15.0 ± 0.7	26.0 ± 0.6
Bacterial strains Gram (-)		
<i>Klebsiella pneumoniae</i> WHO24	na	21 ± 0.9
<i>Pseudomonas aeruginosa</i> ATCC 49189	12.0±0.4	20 ± 1.0
<i>Escherichia coli</i> 25922	14.0±0.6	22 ± 0.8

^a DD: Disc Diameter of inhibition (halo size) in (mm), E. oil 100 µg disc⁻¹.

^b DD: Disc Diameter of inhibition zone of ampicillin (10 µg disc⁻¹), was used as positive control for bacteria. ^d Isolated from *Phoenix dactylifera* (Laboratory of Plant Biotechnology Applied to Crop Improvement, Faculty of Sciences of Sfax, Tunisia). Average±standard deviation were obtained from three different experiments, na: Not active.



products including dextrin and progressively smaller polymers composed of glucose units that cause hyperglycemia and development of type II diabetes mellitus (Güder *et al.*, 2015).

The *in vitro* anti-diabetic assay evaluated the ability of essential oil of *Z. album* to inhibit the activity of α -amylase, a carbohydrate digestive enzyme secreted from the pancreas. Several synthetic drugs are used as potent inhibitors of α -amylase and lipase in the intestine. In this respect, natural α -amylase inhibitors are beneficial in reducing post-prandial hyperglycemia by delaying the digestion of carbohydrates and, consequently, the absorption of glucose. As shown in Table 4, the essential oil of *Z. album* is an interesting pancreatic α -amylase inhibitor with IC_{50} value of $43.17 \mu\text{g mL}^{-1}$. The detected α -amylase inhibitory activity is comparable to that of Acarbose ($IC_{50}= 14.88 \mu\text{g mL}^{-1}$). It should be mentioned that Acarbose (Acar, trade name Glucor) has been used for management of post-prandial hyperglycemia, but it has been reported to be associated with several health side effects (Madar, 1989; Hanefeld, 2007).

Pancreatic Lipase Inhibitory Activity Assay

Obesity is a serious health problem that increases risk for many complications, including diabetes and cardiovascular diseases. It is caused by an excess of caloric intake (Spiegelman and Flier, 2001) and this can be remedied by inhibiting pancreatic lipase activity and by inhibiting or delaying lipid absorption (Ono *et al.*, 2006). The inhibitory effect of essential oil of *Z. album* against pancreatic lipase was compared to that of fluvastatin (IC_{50} of $16.76 \mu\text{g mL}^{-1}$). As shown in Table 5, the IC_{50} value of essential oil ($85.95 \mu\text{g mL}^{-1}$) indicates its good inhibitory activity against pancreatic lipase.

The appreciable anti-diabetic and pancreatic lipase activities of essential oil of *Z. album* could be associated with the presence of phenolic compounds especially eugenol, carvacrol and thymol. In a recent study, we have established that eugenol evidences a powerful anti-diabetic property via inhibition of pancreatic enzyme activities such as α -amylase and lipase (Mnafgui *et*

Table 4. Alpha-amylase inhibition assay of essential oil of *Zygophyllum album*.^a

Sample	Concentration ($\mu\text{g mL}^{-1}$)	% Inhibition	IC_{50} ($\mu\text{g mL}^{-1}$)
Acarbose	25	84.00 ± 2.10	14.88
	50	88.72 ± 2.07	
	100	90.35 ± 1.75	
Essential oil	50	57.95 ± 1.10	43.17
	100	60.43 ± 1.30	
	200	66.90 ± 1.63	

^a Data represent mean \pm SD of three independent experiments.

Table 5. *In vitro* pancreatic lipase inhibition assay of essential oil of *Zygophyllum album*.^a

Sample	Concentration ($\mu\text{g mL}^{-1}$)	% inhibition	IC_{50} ($\mu\text{g mL}^{-1}$)
Fluvastatin	25	74.58 ± 0.74	16.76
	50	86.78 ± 0.83	
	100	93.35 ± 1.03	
Essential Oil	50	36.17 ± 1.03	85.95
	100	58.86 ± 1.27	
	200	85.10 ± 1.59	

^a Data represent mean \pm SD of three independent experiments.

al., 2013). Furthermore, according to McCue et al. (2005), many phenolic compounds such as carvacrol may contribute to additional α -amylase inhibitory activity.

CONCLUSIONS

Our results demonstrated for the first time the potent inhibitory effect of *Zygophyllum album* L. essential oil leaves against key enzymes related to diabetes and obesity. Moreover, this oil is endowed with a moderate antibacterial activity especially against gram-positive bacteria. Furthermore, this investigation showed a notable relationship between phenolic contents and α -amylase, antibacterial, pancreatic lipase inhibitory activities as well as their antioxidant property. Our study provides the possible pharmacologic rationale to the medicinal use of *Zygophyllum album* in the development of antioxidant, anti-diabetic and anti-obesity drugs.

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REFERENCES

- Adams, R. P. 2004. Identification of Essential Oils Components by Gas Chromatography/ Quadropole Mass Spectrometry. Allured Publishing Corporation, Carol Stream III, USA.
- Aderogba, M. A., Okoh, E. K. and Idowu, T. O. 2005. Evaluation of the Antioxidant Activity of the Secondary Metabolites from *Piliostigma reticulatum* (DC.) Hochst. *J. Biol. Sci.*, **5**: 239-242.
- Atta, A. H. and Mouneir, S. M. 2004. Antidiarrhoeal Activity of Some Egyptian Medicinal Plant Extracts. *J. Ethnopharmacol.*, **92**: 303-309.
- Bagamboula, C. F., Uyttendaele, M. and Debevere, J. 2002. Acid Tolerance of *Shigella sonnei* and *Shigella flexneri*. *J. Appl. Microbiol.*, **93**: 479-486.
- Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological Effects of Essential Oils. *Food Chem. Toxicol.*, **46**: 446-75.
- Batish, D. R., Singh, H. P., Kohli, R. K. and Kaur, S. 2008. Eucalyptus Essential Oil as a Natural Pesticide. *Forest Ecol. Manage.*, **256**: 2166-2174.
- Beier, B. A., Chase, M. W. and Thulin, M. 2003. Phylogenetic Relationships and Taxonomy of Subfamily *Zygophylloideae* (Zygophyllaceae) Based on Molecular and Morphological Data. *Plant System. Evol.*, **240**: 11-39.
- Bel Haj Khether, F., Ammar, S., Saidana, D., Daami-Remadi, M., Cheriaa, J., Liouane, K., Mahjoub, M. A., Helal, A. N. and Mighri, Z. 2008. Chemical Composition, Antibacterial and Antifungal Activities of *Trichoderma* sp. Growing in Tunisia. *Ann. Microbiol.*, **58**: 303-308.
- Burt, S. 2004. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods. *Int. J. Food Microbiol.*, **94**: 223-253.
- Ceccarini, L., Macchia, M., Flamini, G., Cioni, P. L., Caponi, C. and Morelli I. 2004. Essential Oil Composition of *Helianthus annuus* L. Leaves and Heads of Two Cultivated Hybrids "Carlos" and "Florom 350". *Ind. Crops Prod.*, **19**: 13-17.
- Dorman, H. J. D., Peltoketo, A., Hiltunen, R. and Tikkanen, M. J. 2003. Characterisation of the Antioxidant Properties of Deodourisation Aqueous Extracts from Selected *Lamiaceae* Herbs. *Food Chem.*, **83**: 255-256.
- Dorman, H. J. D., Surai, P. and Deans, S. G. 2000. *In vitro* Antioxidant Activity of a Number of Plant Essential Oils and Phytoconstituents. *J. Essent. Oil Res.*, **12**: 241-248.
- Duh, P. D., Tu, Y. Y. and Yen, G. C. 1999. Antioxidant Activity of the Aqueous Extract of Harnng Jyur (*Chrysanthemum morifolium* Ramat.). *Lebensmittel-Wissenschaft Technol.*, **32**: 269-277.
- Eskander, E. F. and Won Jun, H. 1995. Hypoglycemic and Hyperinsulinic Effects of Some Egyptian Herbs Used for the Treatment of Diabetes Mellitus (Type II) in Rats. *J. Pharm.Sci.*, **36**: 331-342.



15. Gella, F. J., Gubern, G., Vidal, R. and Canalias, F. 1997. Determination of Total and Pancreatic α -Amylase in Human Serum with 2-Chloro-4-nitrophenyl- α -D-maltotrioside as Substrate. *Clin. Chim. Acta*, **259**: 147-160.
16. Ghoul, J. E., Smiri, M., Ghrab, S., Boughattas, N. A. and Ben-Attia, M. 2012. Antihyperglycemic, Antihyperlipidemic and Antioxidant Activities of Traditional Aqueous Extract of *Zygophyllum album* in Streptozotocin Diabetic Mice. *Pathophysiol.*, **19**: 35-42.
17. Gutiérrez-Larraínzar, M., Rúa, J., Caro, Castro, C. D., Arriaga, D. D., García-Armesto, M. R. and Valle, P. D. 2012. Evaluation of Antimicrobial and Antioxidant Activities of Natural Phenolic Compounds against Foodborne Pathogens and Spoilage Bacteria. *Food Control.*, **26**: 555-563.
18. Güder, A., Gür, M. and Engin, M. S. 2015. Antidiabetic and Antioxidant Properties of Bilberry (*Vaccinium myrtillus* Linn.) Fruit and Their Chemical Composition. *J. Agr. Sci. Tech.*, **17**: 401-414.
19. Hanefeld, M. 2007. Cardiovascular Benefits and Safety Profile of Acarbose Therapy in Prediabetes and Established Type 2 Diabetes. *Germany Cardiovascular Diabetology*, **6**: 20.
20. Hichri, F., Ben Jannet, H., Cheriaa, J., Jegham, S. and Mighri, Z. 2003. Antibacterial Activities of a Few Prepared Derivatives of Oleanolic Acid and of Other Natural Triterpenic Compounds. *Comptes Rendus Chimie*, **6**: 473-483.
21. Jaouhari, J. T., Lazrek H. B. and Jana, M. 2000. The Hypoglycemic Activity of *Zygophyllum gaetulum* Extracts in Alloxan-induced Hyperglycemic Rats. *J. Ethnopharmacol.*, **69**: 17-20.
22. Kaya, G. I., Ünver Somer, N., Konyalıoğlu, S., Yalcin, H. T., Nu, K. G. and Bozkurt, B. 2010. Antioxidant and Antibacterial Activities of *Ranunculus marginatus* Var. *Trachycarpus* and *R. sprunerianus*. *Turk. J. Biol.*, **34**: 139-146.
23. Kavitha, P. and Richard A. H. 2010. Use of Natural Antimicrobials to Increase Antibiotic Susceptibility of Drug Resistant Bacteria. *Int.J. Food Microbiol.*, **140**: 164-168.
24. Keskes, H., Mnafigui, K., Hamden, K., Damak, M., El Feki, A. and Allouche, N. 2014. *In vitro* Anti-diabetic, Anti Obesity and Anti Oxidant Proprieties of *Juniperus phoenicea* L. Leaves from Tunisia. *Asian Pac. J. Trop Biomed.*, **4**: 4649-655
25. Ksouri-Megdiche, W., Medini, F., Mkadmini, K., Legault, J., Magné, C., Abdelly, C. and Ksouri, R. 2013. LC-ESI-TOF-MS Identification of Bioactive Secondary Metabolites Involved in the Antioxidant, Anti-inflammatory and Anticancer Activities of the Edible Halophyte *Zygophyllum album*. *Food Chem.*, **139**: 1073-1080.
26. Madar, Z. 1989. The Effect of Acarbose and Miglitol (Bay-m-1099) on Postprandialglucose Levels Following Ingestion of Various Sources of Starch by Nondiabetic and Streptozotocin-induced Diabetic Rats. *Nutrit. Pharm. Toxicol.*, **119**: 2023-2029.
27. Mastelic, J., Jerkovic, I., Blazevic, I., Poljak-Blazi, M., Borovic, S., Ivancic-Bace, I., Smrecki, V., Zarkovic, N., Brcic-Kostic, K., Vikić-Topic, D. and Muller, N. 2008. Comparative Study on the Antioxidant and Biological Activities of Carvacrol, Thymol, and Eugenol Derivatives. *J. Agric. Food Chem.*, **56(11)**: 3989-3996.
28. McCue, P., Lin, Y. T., Labbe, R. G. and Shetty, K. 2005. Sprouting and Solid-state Bioprocessing by Dietary Fungi Increase the *In vitro* Antibacterial Activity of Aqueous Soybean Extracts against *Helicobacter pylori*. *Food Biotechnol.*, **18**: 229-249.
29. Meng, X. L., Riordan, N. H., Casciari, J. J., Zhu, Y., Zhong, J., Gonzlez, M. J., Miranda-Massari, J. R. and Riordan H. D. 2002. Effects of a High Molecular Mass *Convolvulus arvensis* Extract on Tumor Growth and Angiogenesis. *PR. Health Sci. J.*, **21**: 323-328.
30. Mnafigui, K., Hamden, K., Ben Salah, H., Kchaou, M., Nasri, M., Slama, S., Derbali, F., Allouche, N. and Elfeki, A. 2012. Inhibitory Activities of *Zygophyllum album*: A Natural Weight-lowering Plant on Key Enzymes in High-fat Diet-fed Rats. *Evid.-Based Compl. Altern. Med.*, 1-9. Article ID 620384, doi:10.1155/2012/620384.
31. Mnafigui, K., Kaaniche, F., Derbali, A., Hamden, K., Derbali, F., Slama, S., Allouche, N. and Elfeki, A. 2013. Inhibition of Key Enzymes Related to Diabetes and Hypertension by Eugenol *In vitro* and in Alloxan-induced Diabetic Rats. *Arch. Physiol. Biochem.*, **119**: 1-9.

32. Mnafgui, K., Kchaou, M., Hamden, K., Slama, S., Nasri, M., Ben Salah, H., Allouche, N. and Elfeki, A. 2014. Inhibition of Carbohydrate and Lipid Digestive Enzymes Activities by *Zygophyllum album* Extracts : Effect on Blood and Pancreas Inflammatory Biomarkers in Alloxan-induced Diabetic Rats. *J. Physiol. Biochem.*, **70**: 93–106.
33. Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T. and Shibata, H. 2005. Inhibitory Effects of Oolong Tea Polyphenols on Pancreatic Lipase *In vitro*. *J. Agric. Food. Chem.*, **53**: 4593-4598.
34. Negi, P. S. 2012. Review Plant Extracts for the Control of Bacterial Growth: Efficacy, Stability and Safety Issues for Food Application. *Int. J. Food Microbiol.*, **156**: 7-17.
35. Ono, Y., Hattori, E., Fukaya, Y., Imai, S. and Ohizumi, Y. 2006. Anti-obesity Effect of *Nelumbo nucifera* Leaves Extract in Mice and Tats. *Ethnopharmacol.*, **106**: 238-244.
36. Özkan, A. and Erdoğan, A. 2011. Comparative Evaluation of Antioxidant and Anticancer Activity of Essential Oil from *Origanum onites* (Lamiaceae) and Its Two Major Phenolic Components. *Turk. J. Biol.*, **35**: 735-742.
37. Pérez, C., Agnese A. M. and Cabrera, J. L. 1999. The Essential Oil of *Senecio graveolens* (Compositae): Chemical Composition and Antimicrobial Activity Tests. *J. Ethnopharmacol.*, **66**: 91-96.
38. Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.*, **269**: 337-341.
39. Rajeswara Rao, B. R., Kaul, P. N., Syamasundar, K. V. and Ramesh, S. 2005. Chemical Profiles of Primary and Secondary Essential Oils of Palmarosa (*Cymbopogon martinii* (Rox b.) Wats Var. Motia Burk.). *Ind. Crop. Prod.*, **21**: 121-127.
40. Rimbau, V., Cerdan, C., Vila, R. and Iglesias, J. 1999. Anti Inflammatory Activity of Some Extracts from North African Countries (II). *Phytother. Res.*, **3**: 128-132.
41. Ruberto, G. and Barata M. T. 2000. Antioxidant Activity of Selected Essential Oil Components in Two Lipid Model Systems. *Food Chem.*, **69**: 167-174.
42. Saïdana, D., Mahjoub, S., Boussaada, O., Chriaa, J., Mahjoub, M. A., Chéraif, I., Daami, M., Mighri, Z. and Helal A. N. 2008. Antibacterial and Antifungal Activities of the Essential Oils of Two *Saltcedar* Species from Tunisia. *J. Am. Oil Chem. Soc.*, **85**: 817-826.
43. Salmanian, S., Sadeghi Mahoonak, A. R., Alami, M. and Ghorbani, M. 2014. Phenolic Content, Antiradical, Antioxidant, and Antibacterial Properties of Hawthorn (*Crataegus elbursensis*) Seed and Pulp Extract. *J. Agr. Sci. Tech.*, **16**: 343-354.
44. Sasmakov, S. A., Putieva, M. Zh., Saatov, Z., Kachala, V. V. and Shashkov, A. S. 2001. Triterpene Glycosides of *Zygophyllum eichwaldii* CAM. *Chem. Nat. Compd.*, **37**: 91-92.
45. Sheahan, M. C. and Chase, M. W. 2000. Phylogenetic Relationships within *Zygophyllaceae* Based on DNA Sequences of Three Plastid Regions, with Special Emphasis on *Zygophylloideae*. *Syst. Bot.*, **25**: 371-384.
46. Spiegelman, B. M. and Flier, J. S. 2001. Obesity and the Regulation of Energy Balance. *Cell*, **4**: 531-543.
47. Tepe, B., Sokmen, M., Akpulat, H. A. and Sokmen, A. 2006. Screening of the Antioxidant Potentials of Six *Salvia* Species from Turkey. *Food Chem.*, **95**: 200-204.
48. Teuscher, E. 2006. Medicinal Spices. A Handbook of Vulinary Herbs, Spices, Spice Mixtures and Their Essential Oils. *Med. pharm. GmbH Sci. Pub. Stuttgart*, **29**: 1470-1475.
49. Tigrine-Kordjani, N., Meklati, B. Y. and Chemat, F. 2006. Analysis by Gas Chromatography-mass Spectrometry of the Essential Oil of *Zygophyllum album* L., an Aromatic and Medicinal Plant Growing in Algeria. *Int. J. Arom.*, **16**: 187-191.
50. Tigrine-Kordjani, N., Youcef Meklatib, B. and Chemat, F. 2011. Contribution of Microwave Accelerated Distillation in the Extraction of the Essential Oil of *Zygophyllum album* L. *Phytochem. Anal.*, **22**: 1-9.
51. Yildirim, A., Mavi, A. and Kara, A. A. 2001. Determination of Antioxidant and Antimicrobial Activities of *Rumex crispus* L. Extracts. *J. Agric. Food. Chem.*, **49**: 4083-4089.
52. Yildirim, A., Mavi, A., Oktay, M., Kara, A. A., Algur, O. F. and Bilaloglu, V. 2000.



Comparison of Antioxidant and Antimicrobial Activities of Tilia (*Tilia argentea* Desf. Ex. DC.), Sage (*Salvia*

triloba L.) and Black Tea (*Camellia sinensis* L.) Extracts. *J. Agric. Food. Chem.*, **48**: 5030-5034.

ترکیب شیمیایی و فعالیت های زیستی اسانس *Zygodphyllum album* L. تونس

م. کچائو، ح. بن صلاح، ک. منافگوی، ر. عبدالنبی، ن. غر صلاح، ا. الفکی، م. دمک، و ن. الوچه

چکیده

هدف این پژوهش بررسی فعالیت های آنتی اکسیدانی، آنتی دیابتی، ضد چاقی مفرط، و ضد باکتریایی و نیز تعیین ترکیب شیمیایی اسانس برگ های *Zygodphyllum album* (L.) بود که از منطقه دوز در تونس برداشت شده بود. تجزیه GC-MS مزبور منجر به شناسایی ۱۹ ماده شد که ۹۱/۵۵٪ کل روغن اسانس را تشکیل می داد. مواد اصلی که ۶۳/۶۵٪ کل اسانس را شامل می شد عبارت بودند از *carvacrol* و *cocolactone*، *delta decalactone*، *2,6-Di(tert-butyl)phenol*، *2,2-diphenyl-1-picrylhydrazyl* با استفاده از *Z. album* . فعالیت آنتی اکسیدانی (DPPH)، قدرت کاهیدگی، و سنجش کل ظرفیت آنتی اکسیدانی ارزیابی شد. نتایج به دست آمده آشکار ساخت که اسانس *Z. album* خاصیت قوی ظرفیت آنتی اکسیدانی دارد. افزون بر این، ارزش IC_{50} مربوط به *Z. album* در برابر آلفا آمیلاز ۴۳/۱۷ میکروگرم در میلی لیتر و در برابر لیپاز پانکرات معادل ۸۵/۹۵ میکروگرم در میلی لیتر بود و این امر نشانگر، به ترتیب، اثرات قوی ضد دیابتی و ضد چاقی مفرط بود. همچنین، اسانس *Z. album* اثرات ضدباکتری چشمگیری به ویژه علیه باکتری های گرم مثبت (+) نشان داد. به این قرار، نتایج این پژوهش حاکی از آن است که اسانس *Z. album* را می توان به عنوان منبع طبیعی ماده ای آنتی اکسیدانی، ضد دیابتی و ضد میکروبی در داروها و صنایع غذایی استفاده کرد.