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Original Article

Evaluation of Various Biological Activities of Endemic *Sideritis libanotica* Extracts

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Abstract

In recent years, using of plants to develop combine therapies for treatment of certain diseases such as cancer, Alzheimer's disease, and diabetes mellitus is extremely plausible approach. Perhaps, the definitive treatment of some of these diseases is hidden in the complex content of a plant in nature. Thus, in the present study, we decided to determine the biological activities of methanol and water extracts of the endemic *Sideritis libanotica*. To the best of our knowledge, the present study is the first investigation on antioxidant, enzyme inhibitory activity, antimicrobial activity, and in vitro cytotoxicity of *S. libanotica*. According to results, while the methanol extract shows better anti-cholinesterase, α-glucosidase, and α-amylase inhibition activity and cytotoxicity than the water extract, the tyrosinase inhibitory activity of the water extract was found to be better than the methanol extract. This study provides valuable information on how the biological activity of endemic *S. libanotica* changes in different solvent extractions such as water and methanol.

Keywords: antioxidants; biological activities; enzyme inhibition; *Sideritis libanotica*

Abbreviations: ABTS - 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid); AChE - Acetylcholinesterase; BHT - Butil Hidroksi Toluen; BuChE - Butyrylcholinesterase; CFU - Colony-forming units; ChEI - Cholinesterase inhibitors; DMEM - Dulbecco's modified eagle medium; DMSO - Dimethyl sulfoxide; DPPH - 2,2-Diphenyl-1-picrylhydrazyl; ELISA - Enzyme-linked immunosorbent assay; GC-MS - Gas Chromatography-Mass Spectrometry; MIC - Minimum inhibitory concentration; PBS - Phosphate buffered saline; TTC - 2, 3, 5-Triphenyltetrazolium chloride; USA - United States of America; XTT - (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide).

Introduction

Sideritis species have been widely utilized against gastrointestinal disorders and common colds in folk medicine. So moreover, the pharmacological activities of *Sideritis* species have been attracted many attentions due to their traditional medicine usage and consuming as herbal tea in the world. There are 46 species in the genus *Sideritis* in flora of Turkey and 31 of them are endemic (Davis, 1982; Davis, 1988; Tepe *et al*., 2006; Demirtas *et al*., 2011). *Sideritis* species rich in essential oil, terpenes and flavonoids have been widely consumed for centuries to relieve cold symptoms, including fever, flu, sore throat, bronchitis, and to treat of gastrointestinal disorders such as stomach ache, indigestion and flatulence (Schmeda-Hirschmann and Yesilada, 2005; González Burgos *et al*., 2011; Korkmaz *et al*., 2017a; Korkmaz *et al*., 2017b;) It has been reported that some *Sideritis* species such as *S. lycia* and *S. libanotica* are feared to be in the list of endangered species because of their extensively collection from natural habitat (Kargioglu *et al*., 2008; Polat and Satil, 2012; Dincer *et al*., 2017).

Natural antioxidants especially exist in plants have been extensively studied owing to their beneficial effects on human health. Besides these effects, antioxidants are able to

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maintain nutritional quality and extend the shelf life of food products because of their ability to control rancidity development and retard the formation of toxic oxidation products (Charles, 2013; Sama *et al*., 2014; Patra *et al*., 2016; Yashin *et al*., 2017).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are two types of enzymes that form cholinesterase enzymes. Despite the fact that the AChE and BuChE resemble each other more than 50%, their importance and location are significantly different in the body (Giacobini, 2003; Pohanka, 2011). Because the main function of AChE is rapid splitting of acetylcholine and terminating cholinergic neurotransmission, it is mainly found in many types of conducting tissue such as nerve, muscle, central and peripheral tissues (Koelle, 1954; Chacko and Cerf, 1960; Čolović, 2013). There are two basic types of AChE inhibitors, which are either reversible or irreversible. These inhibitors are used in the diagnosis and/or treatment of some diseases, one of which is Alzheimer's disease (AD). There is no cure for AD, but cholinesterase inhibitors (ChEI) have been used the treat symptoms related to memory, thinking, language, judgment and other thought processes (Koelle, 1954; Chacko and Cerf, 1960; Giacobini, 2004; Lane *et al*., 2006; Čolović, 2013).

Diabetes mellitus (DM) is one of the most prevalent diseases worldwide and millions of people are suffering from this disease. Although glucosidase inhibitors have been used therapeutically since the 1990s, these drugs possess several side effects. For this reason, there is an extremely intensive effort to discover inhibitors with natural and less side effects (Proenca *et al*., 2017; Tundis *et al*., 2010). α-glucosidase and α-amylase are the main starch digestive enzymes and while the α-glucosidase catalyzes the hydrolytic reaction to liberate α-glucose from the non-reducing end of the substrate, α-amylase catalyzes the hydrolysis of α-1,4-glucan bonds in starch maltodextrin and maltooligosaccharides (Truscheit *et al*., 1981; Svensson, 1988; Henrissat, 1998; Kimura, 2000; Yamamoto, 2004; Tundis *et al*., 2010). Today cancer is one of the most dangerous diseases in the world. Therefore, a lot of effort is spent to find a cure for cancer and, the discovery of natural anticancer products as supportive therapy in cancer treatment is of great importance.

The main purpose of this study is to determine the biological activities of methanol and water extracts of the endemic *S. libanotica*. Antioxidant, antimicrobial, cytotoxicity and various enzyme inhibition activities of these two extracts have been studied. Additionally, we identified and compared the chemical content of the extracts. To the best of our knowledge, the present study is the first investigation on antioxidant, enzyme inhibitory activity, antimicrobial activity and in vitro cytotoxicity of *S. libanotica*.

Materials and Methods

Preparation of extracts

The aerial parts of plant were milled with a grinder after dried up to constant weight in shade. Dry plant parts were ground with blender (Blue house). Ten (10) g of plant was soaked in 50 mL of deionized water for 24 h with intermittent shaking. At the end of extraction, it was filtrated by No. 1 Whatman filter paper. The filtrate was concentrated to dryness under reduced pressure with rotary evaporator at 40 °C and this was repeated for three times. The obtained extracts were analyzed by using GC-MS (Sacchetti, 2005). The same procedure was followed for methanol extraction.

In vitro antioxidant activity

The antioxidant activity of the methanol and water extracts of *S. libanotica* leaves was tested using different methods namely as DPPH, ABTS radical scavenging activity, total phenol, and total flavonoid content method.

Determination of DPPH radical scavenging activity

The free radical scavenging activity by methanol extracts was performed according to the method reported by Miser-Salihoglu *et al*. (2013) 150 µL of the extract with different concentration was mixed with 50 µL of 1.0×10^{-3} M freshly prepared DPPH methanol solution in 96 well plate. Methanol was used as control of the experiment. After 30 min of incubation at 25 °C, the reduction of the DPPH free radical was measured reading the absorbance at 517 nm with microplate reader (Epoch, USA). BHT used as positive controls and the percent inhibition was calculated with the following equation:

% Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of control] × 100

Determination of ABTS radical scavenging activity

For determining of ABTS radical scavenging activity of the extracts, followed by the method of Re *et al*. (1999) with slight modification. The stock solution of ABTS was made by reacting 7 mM ABTS solution with 2.4 mM of potassium persulfate solution in equal volume for 16 h. working solution was then prepared by diluting the stock ABTS solution with methanol to give an absorbance of 0.7 ± 0.02 units at 734 nm using a microplate reader (Epoch, USA). In each experiment, the ABTS solution was prepared freshly. 50 µL of extract was mixed with 150 µL ABTS working solution and stand for 10 min at dark place. All the analyses were conducted in triplicate and the results expressed as mean ± standard deviation. Appropriate blanks (methanol) and standard (BHT) were run simultaneously.

Determination of total phenolic contents (TPC)

To measure the total phenolic content in the extracts, spectrophotometric Folin–Ciocalteu method was used as previously described by Clarke *et al*. (2013) with slight modification. Briefly, 20 µL of extract in DMSO was mixed with 100 µL freshly 1/10 diluted F-C reagent with distilled water. After five min, the solution was mixed with 80 µL of 7.5% Na_2CO_3 solution, and incubated for 30 min at 25 °C. The measurement of absorbance was done at 650 nm in a microplate reader (Epoch, USA). All the analyses were performed in triplicate and the results expressed as mean ± standard deviation. Appropriate blanks (DMSO) and standard (gallic acid in DMSO) were run simultaneously, after which the total phenolic content (TPC) was calculated as milligrams gallic acid equivalents per gram of dry extract.

Estimation of total flavonoid content (TFC)

For determination of total flavonoid content, the aluminum chloride colorimetric method was used as previously described by Molan and Mahdy (2014) using catechin as the reference standard. Briefly, 25 µL of 1 mg/mL test sample solution, 100 µL of dd. H_2O and 7 µL of 5% $NaNO_2$ were mixed together in 96-well plates. After 15 min of incubation at room temperature, 7 µL of 10% AlCl3 was added. After 5 min, 50 µL of 1 M NaOH and 60 µL of distilled water were added to each well. Then the absorbance was measured at 490 nm in a microplate reader (Epoch, USA). All determinations were carried out in triplicates. The content of total flavonoids was expressed as mg of catechin equivalent per g of dry weight of extract.

Enzym activities

Acetylcholinesterase/butyrylcholinesterase inhibition assay

Experiment was carried out according to the Ellman *et al*. (1961) method. The assay was performed in the following way. The mixture consisted of 20 µL of test sample/reference standard of various concentrations, 140 µL of 0.1 mM phosphate buffer (pH 6.8), 10 µL of 3 mM 5,5'-dithio-bis-nitrobenzoic acid (DTNB) and 20 µL of enzyme (0.22 U/mL for acetylcholinesterase/ 0.1 U/mL for butyrylcholinesterase) prepared in phosphate buffer was incubated for 5 min at 25 °C. Following preincubation, 10 µL of the substrate (0.71 mM acetylthiocholine iodide/0.2 mM butyrylthiocholine chloride in phosphate buffer) was added to start the reaction and incubated again for 10 min. The developed yellow color was measured at 412 nm (Epoch, USA). Galanthamine was used as positive control.

α-Glucosidase inhibition activity

α-Glucosidase inhibition method was followed by Kumar *et al*. (2012). Acarbose was used as a positive control, while phosphate buffer was used as negative control in place of sample. Each concentration was carried out in triplicate. 25 µL of sample solution diluted with buffer was mixed with 25 µL of α-glucosidase (0.5 U/mL), incubate for about 10 min at 25 °C. Then 25 µL of 0.5 mM 4-nitrophenyl-β-D-glucoronide (pNPG) was added to each well as substrate and incubated further 30 min at 37 °C. After incubation period, 100 µL of 0.2 M sodium carbonate was added to terminating the reaction and the absorbance was read at 405 nm.

α-Amylase inhibition activity

α-Amylase inhibition method was followed by Kumar *et al*. (2013). Acarbose was used as a positive control, while phosphate buffer (0.02 M PBS, pH 6.9) was used as negative control in place of sample. Each sample was carried out in triplicate with different concentrations. The reaction mixture containing 50 µL of sample solution diluted with buffer, 25 µL of α-amylase from porcine pancreases (0.5 mg/mL) incubated for about 10 min at 25 °C. Then 50 µL of 0.5% starch solution (w/v) prepared freshly was added to each well as substrate and incubated further 10 min at 25 °C. After incubation period, 100 µL of 1% 3, 5-dinitrosalicylic acid (DNS) color reagent was added as color reagent and heated at water bath for 10 min. The absorbance was read at 540 nm.

Tyrosinase inhibition activity

Tyrosinase inhibitory activity was determined by the 96-well plate spectrophotometrically as described by Jeong *et al*. (2009) with slight modifications. Kojic acid was used as a positive control, while phosphate buffer (0.1 M PBS, pH 6.8) was used as negative control in place of sample. Each sample was performed in triplicate. Briefly, 20 µL of sample solution with different concentrations was added to a 96-well microplate and mixed with 20 µL of tyrosinase from mushrooms (250 U/mL). After 10 min of incubation at 25 °C, 20 µL of 3 mM L-tyrosine was added to each well as substrate and incubated further 30 min at 25 °C. After incubation period, the absorbance of the reaction mixture was read at 492 nm in a microplate reader (Epoch, USA).

Determination of minimum inhibitory concentration (MIC) of Sideritis libanotica extracts

The minimum inhibitory concentration (MIC) of the *S. libanotica* water and methanol extracts against bacteria and fungus was carried out by broth microdilution method using 96-well cell culture plates (Eloff, 1998). For this purpose, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 11778), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (DSM 11953) strains were used. The extracts were dissolved in Dimethyl Sulfoxide (DMSO) at 50 mg/mL concentration. Ten (10) µl samples were added in the first line of a microtiter plate which was diluted with 90 µl broth. Next, 50 µl sample was added in the second line of a microtiter plate which was serially diluted two-fold with broth. Concentration of the extracts in wells ranged from 2.5 to 0.004 mg/mL. Column 11 and 12 was used as a negative control for medium sterility (no microorganism) and positive growth control (no extracts), respectively.

Mueller Hinton Broth (Accumix® AM1072) was used for bacteria and Saboraud Dekstroz Broth (Himedia ME033) for Candida. Bacteria and fungi suspension (50 µL) were added on prepared samples. Final inoculum size was 5 $x10^5$ CFU/mL at bacteria and 0.5-2.5 $x10^3$ CFU/mL at Candida every well (CLS, 2002; CLSI, 2012). Samples, which added bacteria, were incubated at 37 °C and samples that added Candida were incubated at 35 °C between 16-24 hours. Afterwards, 40 µL (2 mg/mL) 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) was added to each well to indicate microbial growth. The microtiter plates were further incubated at 37 °C for 2 h.

In vitro cytotoxicity assay

Cell lines and reagents

Human breast adenocarcinoma cell line, MDA-MB-231 (HTB-26) and mouse fibroblast cells, L929 (CRL-6364) were purchased from American Type Culture Collection (ATCC, Manassas, VA). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and sterile phosphate buffer saline (PBS) were purchased from PAA Ltd. (France). Trypsin-EDTA was supplied from Biological Industries Ltd. (Haemek, Israel). L-glutamine–penicillin–streptomycin solution was from Sigma-Aldrich (Steinheim am Albuch, Germany). XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Roche Diagnostic.

Cell culture

The cytotoxicity of the *S. libanotica* methanol and water extracts was tested against MDA-MB-231 and L929 cell lines. Both cell lines were cultured in DMEM containing with 10% FBS, 1% L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin in 25 cm^2 polystyrene flasks and maintained in a humidified atmosphere with 5% CO_2 at 37 °C. Growth and morphology were monitored, the culture medium was changed every two days, and cells were passaged when they had reached 80-90% confluence.

Cell viability assay

The antiproliferative activity of the *S. libanotica* methanol and water extracts was evaluated using the XTT colorimetric assay. The extracts were dissolved in DMSO and diluted in DMEM prior to treatment. Initially, cancer and control cells were seeded at a density of $1x10^4$ cells per well in 96-well culture plates in 100 µL of culture medium and were allowed to attach overnight before treatment. The next day, these cells were treated with serial concentrations (0.0625, 0.125, 0.25, 0.5, 1 mg/mL) of *S. libanotica* for 24 h. Besides, non-treated cells and cells treated with DMSO (0.5%) were used as negative control and solvent control respectively. After that, the treatment medium was removed and wells were washed twice with 200 µL phosphate buffered saline (PBS). At the end of these periods, for determination of living cells, 100 µL DMEM without phenol red and 50 µL XTT labeling mixture were added to each well and then the plates were incubated for another 4 h. The absorbance of XTT-formazan was measured using micro plate reader (Thermo, Germany) at 450 nm against the control (the same cells without any treatment). All experiments were performed in three independent experiments and the cell viability was expressed in % related to control (100% of viability).

Statistical analysis

Statistical analysis was performed using the Graphpad prism 7.0 (USA, Sandiago) software. On antioxidant, anti-AchE, Anti-BchE, α-glucosidase and α-amylase and tyrosinase inhibitory activity results were expressed as mean ± standard deviation (SD) of the mean of three parallel measurements. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test or Tukey's Multiple Comparison test. The $P < 0.05$ was considered to indicate statistical significance.

Results and Discussion

The chemical contents of the extracts were determined by using the GC-MS (Table 1). As seen in Table 1, the chemical contents of the methanol and water extracts showed significant differences. In fact, this difference accounts for the significant changes in the biological activities of the extracts. These changes in biological activity are briefly discussed below.

Antioxidant activity

The *in vitro* antioxidant activities including total phenolic and flavonoid contents, DPPH and ABTS radical scavenging activities of *S. libanotica* in the methanol and water extract were investigated and compared with the standard antioxidant compounds BHA and gallic acid.

Total phenolic and flavonoid content

As shown in Table 2, the total phenolic content values for the water and methanol extract of *S. libanotica* range from 62.79 ± 1.06 to 111.53 ± 8.3 mg gallic acid equivalents (GAE)/g dried extract, while their total flavonoid content values range from 25.45 ± 3.18 to 43.56 ± 0.60 mg catechine equivalents (CE)/g dried weight of extract. The methanol extract possess higher TPC and TFC values than the water extract. In other words, the methanol extracts have more phenolic compounds and flavonoids than the water extract. Quantitative analysis of total phenolics and flavonoids in different extracts (ethanol, diethylether, ethyl acetate and n-butanol) of *Sideritis scardica* was performed previously by Tadic *et al.* (2012), and the ethyl acetate extract was found contain highest amount of total phenolic (345.6 mg GAE/g DW) and flavonoid content (1.1%). In another study by Deveci *et al.* (2017) the total phenol and flavonoid content of *Sideritis pisidica* Boiss. Hexan, methanol and acetone extracts were found between 20.40 - 100.24 mg pyrocatechol equivalents per gram and 30.66 - 43.75 mg quercetin equivalents per gram, respectively (Deveci *et al.*, 2017). According to these results, the total phenol and flavonoid content of *S. libanotica* was found between those results.

DPPH and ABTS radical scavenging activity

In terms of DPPH and ABTS radical scavenging activity, the extracts showed a concentration-dependent inhibitory activity and results are given in Table 2. The methanol extract exhibited the highest overall DPPH and ABTS free radical scavenging capacity, the IC50 value was determined as 49.62 ± 3.55 µg/mL and 39.52 ± 1.27 µg/mL, respectively. In addition, extremely high DPPH and ABTS radical scavenging activity difference is observed between the methanol and the water extracts probably due to the excess of soluble in methanol extracts.

Enzym activities

Anti-cholinesterase activity

When the anti-acetylcholinesterase and anti-butyrylcholinesterase inhibition activities of the methanol and water extract of *S. libanotica* were compared with each other, the methanol extract was found to be more active than the water extract. As it seen presented in Table 2, the methanol and water extract displayed 73.45 ± 3.50% and 65.58 ± 2.47% inhibition on acetylcholinesterase and 31.48 ± 3.74% and 27.6 ± 1.06% butyrylcholinesterase inhibition activity. The high amounts of phenolic and flavonoids found in the methanol extract may be responsible for the anticholinesterase activity. Although the methanol extract shows strong anti-acetylcholinesterase and anti-butyrylcholinesterase inhibition activity, it still has lower activity values than the reference drug.

α-Glucosidase and α-Amylase inhibition activity

The inhibitory activities of the methanol and water extract of *S. libanotica* were evaluated against α-glucosidase and α-amylase enzyme (Table 2) in comparison with the positive control drug acarbose. Previous studies reported that the flavonoids with hydroxyl group have the inhibition activity to α-glucosidase enzyme (Salah *et al.*, 2017).

Table 1. Chemical composition of water and methanol extracts of *S. libanotica*

Chemical Component	RT	Water	Methanol
Cyanomethyl 2-chloroethyl sulfide	4.294	1.17	
1H-Pyrrole, 1-methyl-	4.695	0.52	
Tetrahydrothiophen-3-one	5.433		0.11
2-Furanmethanol (CAS)	6.726	0.20	
4-Cyclopentene-1,3-dione	7.521	0.37	
isomeric dihydro - methyl - furanone	8.980	2.41	
1,2-Cyclopentanedione	9.026		0.28
Phenol (CAS)	11.406	0.25	
2,4,6(1H,3H,5H)-Pyrimidinetrione (CAS)	14.250	0.49	
Phenol, 2-methoxy- (CAS)	15.046	0.27	
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	16.768	1.57	
1,4:3,6-Dianhydro-. alpha. -d-glucopyranose	18.668		0.12
2,3-DIHYDRO-BENZOFURAN	19.034		0.20
2-Methoxy-4-vinylphenol	22.605		0.59
4-vinyl-2-methoxy-phenol	22.622	1.89	
n-Tridecan-1-ol	28.275		2.05
1-Dodecanol	28.281	7.69	
Phenol, 2,4-bis(1,1-dimethylethyl) - (CAS)	29.379		0.75
Phenol, 2,4-bis(1,1-dimethylethyl)	29.391	3.25	
Megastigmatrienone	32.166	0.89	
Cyclododecane	33.470	4.62	
Dodecyl acrylate	33.476		4.94
Lidocaine	37.476		0.42
Hexadecanoic acid, methyl ester	37.739	5.89	0.67
n-Hexadecanoic acid	38.380	0.93	
1,15-Hexadecadiene	39.971		0.45
6,9,12-Octadecatrienoic acid, methyl ester	40.217	1.57	
9,12-Octadecadienoic acid (Z, Z)-, methyl ester (CAS)	40.457	5.51	
10-Octadecenoic acid, methyl ester (CAS)	40.543	3.61	
Methyl stearate	40.920	1.25	
3-Ethyl-4,6-dimethylpyrano[3,2-g] chromene-2,8-dione	43.570		0.55
9-Octadecenamide, (Z)-	44.394	18.38	
Octadecane	46.047		0.66
4-Hydroxymethyl-3-(2',4'-dimethyl-7'-indolyl)-2-methylindole	47.541		64.98

Table 2. *In vitro* antioxidant and enzyme inhibitory activities (%) of the methanol and water extracts from aerial parts of *S. libanotica*

Extracts	DPPH Radical Scavenging Activity	ABTS Radical Scavenging Activity	Total Phenolic Content	Total Flavonoid Content	Anticholinesterase Activity*		Antidiabetic Activity*		Skin Whitening
					AChE	BChE	α-Glucosidase	α-Amylase	Tyrosinase
Methanol	49.62 ± 3.55	39.52 ± 1.27	111.53±8.3	43.56± 0.60	73.4± 3.50	31.4±3.74	32.87± 0.89	36.34± 3.91	58.21± 2.72
Water	685.81 ± 1.25	211.96 ± 1.41	62.79 ± 1.06	25.45 ± 3.18	65.5± 2.47	27.6 ± 1.06	25.93 ± 1.61	14.08 ± 1.71	75.32 ± 3.25
Reference Drugs									
Galanthamine Hydrobromide					93.87± 0.56	89.89 ± 0.01			
Acarbose							57.56 ± 0.52	58.40 ± 0.63	
Kojic Acid									56.42 ± 1.59

*at 2 mg/mL concentrations

The same with anticholinesterase activity, the methanol extract demonstrated higher inhibitory activity both to α-glucosidase (32.87 ± 0.89%) and α-amylase (36.34 ± 3.91%) enzyme than the water extract. However, the activity was lower than the reference drug acarbose (57.56 ± 0.52% and 58.40 ± 0.63%, respectively).

Tyrosinase inhibition activity

In recent years, tyrosinase inhibitors have gained importance due to the effect of tyrosinase enzyme on human health, browning of foods and the role of determine skin tone. In the method used, L-tyrosine was used as the substrate to determine the phenolase activities of mushroom tyrosinase, while kojic acid was used as the reference drug. The tyrosinase inhibitory activities of the methanol and water extracts of *S. libanotica* are presented in Table 2 as percentage at the concentration of 2 mg/mL. Our results revealed that the water extract showed a good tyrosinase inhibitory activity than the methanol extract, however, it was higher than the kojic acid which was used as a positive control showed an inhibition level of 56.42%. Deveci et al reported that, the methanol (23.29 ± 0.56%) and acetone (15.66 ± 0.11%) extracts of *Sideritis stricta* were found to be low active against tyrosinase, while the hexane and water extracts were found to be inactive (Deveci *et al*., 2018).

To the best of our knowledge, the present study is the first investigation on antioxidant and enzyme inhibitory activity of *S. libanotica*. The obtained results showed that the methanol extract that possessed better antioxidant, anti-acetylcholinesterase effects. In addition, the methanol extract showed higher amount of total phenolic and flavonoid content than water extract. Generally, the higher antioxidant activity of methanol extract of *S. libanotica* extract than water extract can be correlated primarily with their higher amount of total phenolic and flavonoid contents. Therefore, further phytochemical and bioactivity-guided isolation of *S. libanotica* could be carried out to identify their active compounds.

Antimicrobial activity

The antimicrobial activity of plant extracts are accepted significant if the MIC value is 0.1 mg/ml or lower, moderate if 0.1 < MIC ≤ 0.625 mg/ml and weak if MIC > 0.625 mg/ml (Kuete, 2010; Awouafack *et al*., 2013). The results of the microdilution broth assay showed that water extracts of *S. libanotica* appeared to have weak antimicrobial activity against all the tested microorganisms (MIC values of 2.5 - >2.5 mg/mL). MIC values of the methanol extract against *S.aureus* and *B.cereus* were found different the MIC value of the water extract (0.156 mg/mL). The methanol extract of *S. libanotica* appeared to have moderate antimicrobial activity against *S.aureus* and *B.cereus*. MIC values of the water and methanol extract against other tested microorganisms were observed similar results (Table 3).

Cell viability

XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) cell proliferation assay was used to evaluate the antiproliferative effects of the water and methanolic extracts of *S. libanotica* on MDA-MB-231 and L929 cell lines. As shown in Fig. 1, the methanol extract at 0.5 and 1 mg/mL concentration statistically significant inhibited MDA-MB-231 cell proliferation ($p<0.05$) in a dose-dependent manner (IC50 = 0.67 mg/mL). On the other hand, it has been found that the water extract has no cytotoxic effect at any concentration ($p>0.05$). Furthermore, neither extract exhibited any significant cytotoxicity on the L929 cell line at the concentrations range (1-0.0625 mg/mL).

Our cytotoxicity results evidently showed that the methanol extract is more toxic than the water extract of *S. libanotica*. This may be because the methanol extract has richer active ingredients than the water extract, as shown in Table 1. Additionally, the anticancer effects may be associated with antioxidant features due to its polyphenolic components quantity (Fig. 1).

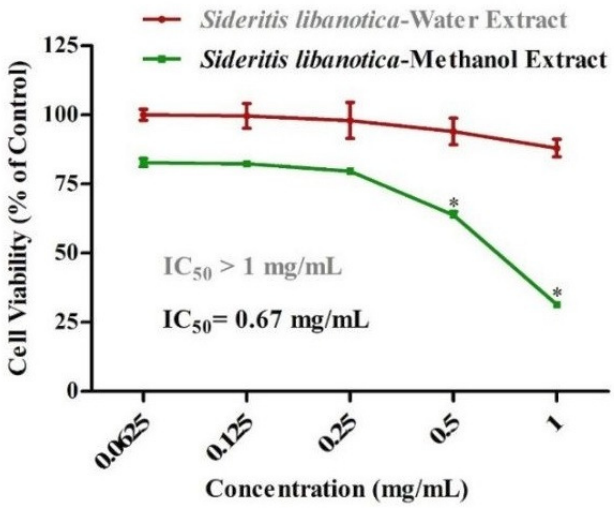

Fig. 1. Effects of the water and methanol extracts from *S. libanotica* on the viability of MDA-MB-231 cell line, after treatment with various concentrations (range: 0.065-1 mg/mL) for 24 h. Both extracts show no toxicity in normal cells

Table 3. Antimicrobial activity results of S. libanotica extracts

	Microorganisms and MIC Values (mg/mL)					
	E.coli	*S.aureus*	*P.aeruginosa*	*B.cereus*	*C.albicans*	*C.tropicalis*
	ATCC 25922	ATCC 29213	ATCC 27853	ATCC 11778	ATCC 10231	DSM 11953
Water Extract	2.5	2.5	2.5	2.5	>2.5	2.5
Methanol Extract	2.5	0.156	2.5	0.156	>2.5	2.5

Conclusions

In the present study, some biological activities including antioxidant, cytotoxicity, antimicrobial and various enzyme inhibition activities of the methanol and water extracts of the endemic *S. libanotica* have been investigated. It is also demonstrated the chemical content of extracts by using the GC-MS. The higher antioxidant activity of the methanol extract of *S. libanotica* can be correlated to the presence of more flavonoids and phenolic compounds of the methanol extracts than the water extracts. Additionally, the methanol extract shows better anti-cholinesterase activity and α-glucosidase and α-amylase inhibition activity than the water extract. However, an opposite situation was observed for tyrosinase inhibitory activities, i.e. the tyrosinase inhibitory activity of the water extract was found to be better than the methanol extract. Despite the intensive use of *Sideritis* species to relieve cold symptoms, antimicrobial efficacy of *S. libanotica* was found to be lower than expected. According to the cytotoxicity results, the methanol extract found to be more toxic than the water extract of *S. libanotica*. In conclusion, this study provides valuable information on how the biological activity of endemic *S. libonitica* changes in different solvent extractions such as water and methanol.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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