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COMPARISON BETWEEN THE CHEMICAL COMPOSITIONS AND THE *IN-VITRO* ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITIES OF *SALVIA LIBANOTICA'* AND *SALVIA OFFICINALIS'* LEAVES ESSENTIAL OILS

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ABSTRACT

Salvia (Sage) species have drawn a great attention of researchers all around the world due to their countless benefits to support their traditional uses and to find new biological effects. These properties are affected by several environmental factors, which may explain the differences in the magnitude of the reported biological effects between cultivation regions. The purpose of this study was to cultivate *S. officinalis* in Lebanon and to collect Lebanese endemic *S. libanotica* to extract their leaves' essential oil (EO) by hydrodistillation using the Clevenger apparatus, to determine the chemical composition, and to evaluate the antidiabetic and anti-inflammatory activities of their EOs using the α-glucosidase and the albumin denaturation inhibition assays, respectively. *S. officinalis* (1.75% w/w) had a higher EO yield than *S. libanotica* (0.86% w/w). Eucalyptol and camphor were the main components detected in the two species EO. *S. officinalis* exhibited an anti-diabetic effect at lower concentrations (5 and 10%) than *S. libanotica*, however, both species induced at the highest concentration used (75%) a similar α-glucosidase inhibition. Furthermore, the anti-inflammatory effect of the two species was not statistically different at low concentrations (1, 2.5, and 5%), however, at the concentrations of 10 and 15%, *S. libanotica* still had a residual anti-inflammatory effect whereas *S. officinalis* did not. The current study demonstrated early evidence of distinguished anti-diabetic and anti-inflammatory effects induced by the EO of Lebanese *S. officinalis* and *S. libanotica*.

KEYWORDS: *Salvia officinalis, Salvia libanotica,* essential oil, antidiabetic, and anti-inflammatory effects.

INTRODUCTION

Salvia (Sage) species have been cultivated since ancient times by different communities and regions as a culinary herb, an ornament or for healing purposes in folk medicine. This genus is the largest member in the mint family (Lamiaceae / Labiatae) comprising over 900 species distributed throughout the world.[1,2] Species from this genus, such as *S. officinalis* and *S. libanotica,* have been subject of intensive study in the past decades for their biological activities, including the hypoglycemic and anti-inflammatory effects in relation to their active constituents, such as the flavonoid and phenolic contents.[3–5]

Salvia libanotica, (also named *S. fruticosa* and *S. triloba*), native to the Mediterranean region, is predominantly found in Lebanon where it grows wildly in different sites. It is still regarded as a traditional remedy against many ailments, giving it its popularity and importance in Lebanon.[6,7] The major components of its EO have been reported to be 1,8-Cineol, β-Pinene, Terpineol and Caryophyllene.[8] In Lebanese folk medicine, *S. libanotica* is widely used for its anti-diabetic properties in spite of the scarcity of scientific evidence of its efficacy in the literature.[4] A study demonstrated that the chronic intake of *S. libanotica* infusion helps in the prevention of high fat-induced hyperglycemia and dyslipidemia in rats by increasing fasting serum insulin and liver glycogen content compared with controls.[9] Moreover, the hypoglycemic effects of *S. libanotica* has been reported when using a 10 % infusion of the plant leaves at an oral dose of 250 mg of dry leaves/kg body weight in alloxan-induced diabetes in rabbits mainly by reducing intestinal absorption of glucose.[10] Regarding its anti-inflammatory effect, extracts of *S. libanotica* have been shown to suppress in Wistar rats both acute inflammation (noted by a decrease in edema size) and chronic inflammation (noted by a decrease in the mean of pellet weight) compared to the control.[11]

Salvia officinalis L. (also known as common sage) is a perennial, evergreen subshrub which can reach up to 60 cm in height. This plant is also native to the

Mediterranean region and nowadays, it has been naturalized throughout the world.[1] Between *Salvia* species, *S. officinalis* is known to have the highest essential oil yield (up to 3%). Its essential oil is a complex mixture usually rich in many biologically active compounds mainly α- and β-thujone, camphor, 1,8-cineole and borneol, α-pinene, α-humulene and β-caryophyllene.[12,13] Several studies have investigated the potential anti-diabetic properties of different extracts of *Salvia officinalis* collected from different regions.[14–16] For instance, the treatment of diet-induced obese (DIO) mice with Tunisian methanol extract of *S. officinalis* (100 and 400 mg kg^{-1}/day bid), or rosiglitazone (3 mg kg−1 /day bid), showed that methanol extract at the lowest dose exhibits similar effects to rosiglitazone. It improves insulin sensitivity, inhibits lipogenesis in adipocytes and reduces inflammation as judged by plasma cytokines.[17] In another study, drinking of sage tea (300 ml, twice a day) showed an increase in antioxidant defenses and improved the lipid profile, without causing any hepatotoxicity or inducing any adverse effects such as changes in blood pressure, heart rate, and body weight, which may indirectly improve the diabetic condition.[18] The anti-inflammatory effect of *S. officinalis*' EO and extracts has been demonstrated in several studies. For instance, *S. officinalis* leaf extracts have been shown to suppress in a dose-dependent manner both acute inflammation (evaluated by a decrease in edema size) and chronic inflammation (evaluated by a decrease in the granuloma tissue formation) compared to the control group.[19] In another study, *S. officinalis*' EO significantly inhibited in lipopolysaccharide- stimulated mouse macrophages the nitric oxide production which plays a key role in the pathogenesis of inflammation.[20]

It has been mentioned that several environmental factors (such as soil moisture, climatic conditions, daylength etc.) may influence the quantitative and qualitative compositions of essential oils, leading to differences in the magnitude of the biological effects between regions.[15,21–23] For instance, the Gas chromatography/Mass spectrometry (GC/MS) analyses of the essential oil (EO) of *Salvia officinalis* cultivated in Tunisia revealed that the amount of characteristic components (such as α-Thujone, β-Thujone, Camphor and 1,8-Cineole) obtained from the latter's oil were similar to those in collected species from Macedonia,[24] and South of Brazil,[25] but were different from Iranian,[26] Lithuanian,[27] German,[28] and Egyptian,[29] ones.

In Lebanon, limited research has been conducted on a few representatives of *Salvia* genus, particularly the endemic *S. libanotica*, nevertheless, since the Lebanese flora does not include *S. officinalis*, and in light of the impact of environmental factors, we have cultivated *S. officinalis* in Lebanon. Then, we investigated the chemical composition and the *in-vitro* effect of the EO extracted from the leaves of Lebanese-cultivated *S. officinalis* and -endemic *S. libanotica* on diabetes and inflammation, which has an emerging role in diabetes pathophysiology and whose targeting has generated increasing interest to improve prevention and control of the disease, using the α-glucosidase and albumin denaturation inhibition assays, respectively.

I. MATERIALS AND METHODS

I.1 Collection of plant material

Commercially available seeds of *Salvia officinalis* were purchased from Royal Sluis, Italy and were cultivated in a plant nursery in Lebanon. Leaves from Lebanese- wild growing *Salvia libanotica* and Lebanese- cultivated *Salvia officinalis* were collected during the pre-flowering stage. After collection, the leaves were dried at room temperature and properly stored until the oil extraction.

I.2 Extraction of the essential oil

The EOs from dried *S. libanotica'* and *S. officinalis'* leaves were obtained by hydro-distillation procedure using a Clevenger-type apparatus. Briefly, 30g of *S. libanotica'* and *S. officinalis'* dried leaves were subjected to hydro-distillation using distilled water as a solvent. The extraction lasted for 3 hours after which the oil volume in the burette was collected and stored in the refrigerator until usage.

I.3 Gas-chromatography/Mass spectrometry analysis (GC/MS)

Fifty µl of volatile oil sample were diluted to 250 µl by hexane. One µl from this solution was then used for *Gas Chromatography (GC)* analysis. GC analysis was carried out on a thermoscientific (GC) with mass spectrometer detector and a SLB 5 MS capillary column (30m × 0.25 mm; film thickness 0.25 µm). The carrier gas was helium with a flow rate of 0.7 ml/min. The oven temperature for the first 4 min was kept at 60 ˚C and then increased at a rate of 5˚C/min until reached to the temperature of 250˚C and kept on for 5 minutes. The injector and detector temperature were set at 250˚C. The data output from the detector appeared as a line graph (chromatogram), with the amount of compound detected shown against the retention time. The volatile compounds appeared as peaks on the graph. The relative abundance for each component is thus calculated as follows:

$$\%\ \text{Relative Abundance} = \frac{\text{Area for each component}}{\text{Sum of Areas}} \times 100$$

I.4 Alpha-glucosidase inhibition assay

Alpha-glucosidase inhibitory activity was determined using a 96-well microtiter plate with PNPG as the substrate according to the method described by Rengasamy *et al* (2013), with some modifications as described by Yani *et al* (2018)[30,31] Briefly, different concentrations of *S. libanotica'* and *S. officinalis* leaves' EOs were prepared by dilution in DMSO 2% into a final volume of 200 µL. After that, 50 µL of the oil were placed in their corresponding ELISA well and 50 µL of 1

U/mL of α-glucosidase were added to each well. The ELISA plate was left for incubation at room temperature for 10 minutes. A negative control (50 µL DMSO 2% added to 50 µL α-glucosidase) and a positive control (50 µL of Acarbose at 20 mg/mL added to 50 µL of α-glucosidase) were also prepared and treated similarly to determine their absorbance. After the incubation, 50 µL PNPG were added to each of the wells. Finally, the reaction was stopped by the addition of Glycine-Sodium Hydroxide buffer (pH 10.8) and the absorbance was measured at 405 nm by using a microplate reader. The experiment was performed in triplicate.

The percentage of α-glucosidase inhibition was calculated as follows

$$\text{Percentage of enzyme inhibition (\%)} = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} x\ 100$$

I.5 Albumin denaturation inhibition assay

Protein denaturation assay was conducted following a slightly modified method described by Uriah *et al* (2019).[32] Briefly, different concentrations (ranging between 1% and 20%) of *S libanotica'* and *S. officinalis* leaves' EOs were prepared by dilution in distilled water into a final volume of 150 µL. Then, a 1% w/v aqueous solution of Bovine Serum Albumin (BSA) was prepared. After that, 50 µL of the oil were placed in their corresponding tubes and 450 µL of BSA were added to each tube. The tubes were heated in a water bath at 37°C for 20 minutes and then at 57°C for 10 minutes to denature the albumin protein. A blank (3 mL of distilled water), a negative control tube (50 µL distilled water added to 450 µL BSA) and a positive control (50 µL of Sodium Diclofenac at 250 µg/mL added to 450 µL of BSA) were also prepared and treated similarly to determine their absorbance. After heating and cooling at room temperature, 2.5 mL of Saline Phosphate buffer with a pH of 6.4 were added to each tube. Finally, the absorbance was measured at 660 nm using a UV–visible spectrophotometer. The experiment was performed in triplicate.

The percentage of albumin denaturation inhibition was calculated as follows

$$\text{Percentage of denaturation inhibition (\%)} = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} x\ 100$$

I.6 Statistical analysis

All experiments were performed at least three times in triplicates. The results were expressed as mean value ± SEM. Non-parametric tests (Kruskal-Wallis followed by Mann Whitney tests) were used to compare groups using the SPSS software version 22.0. A value is considered significant if $P \leq 0.05$.

II. RESULTS

III.1 Essential oil yield

The yields of EOs (%w/w) obtained from *S. libanotica'* and *S. officinalis'* leaves from Lebanon were determined by the following equation

$$Yield\ of\ essential\ oil\ (\%) = \frac{mass\ of\ the\ EO\ (g)}{mass\ of\ the\ dried\ leaves\ (g)} x\ 100$$

Hydro-distillation of air-dried leaves of *S. officinalis* cultivated in Lebanon yielded 1.75% (w/w on a dry weight basis) of essential oil with yellow color and strong pleasant smell. In contrast, Lebanese endemic *S. libanotica* yielded 0.86% (w/w on a dry weight basis) of EO.

III.2 Chemical composition

The GC/MS analysis of each of *S. libanotica*'s and *S. officinalis'* EO revealed the presence of eighteen and twenty components, respectively (Table 1).

Among the twenty components identified in the EO of *S. libanotica* five were found predominately high, with a relative abundance higher than 3%. These were eucalyptol (64.27%), caryophyllene (10.59%), alpha-pinene (7.77%), camphor (5.28%) and 1R-alpha-pinene (3.82%).

On the other hand, among the eighteen components found in the EO of *S. officinalis*, alpha-thujone (38.56%), camphor (29.53%), eucalyptol (14.64%), alpha-humulene (5.92%), and camphene (5.53%) were the predominant components.

Table 1: Chemical composition of Lebanese- endemic *S. libanotica* and -cultivated *S. officinalis* leaves' EOs.

Component name / Relative abundance (%)*	*Salvia libanotica*	*Salvia officinalis*
1R-alpha-Pinene	3.82	-
Alpha-Pinene	7.77	-
Eucalyptol (1,8-cineole)	64.27	14.64
Gamma-Terpinene	0.56	0.25
Alpha-Thujone	0.97	38.56
Camphor	5.28	29.53
p-Menth-1-en-8-ol	0.94	-
Isobornyl acetate	0.12	-
Camphene	0.18	5.53
Neoclovene	0.07	-
Caryophyllene	10.59	-
Aromadendrene	1.15	1.50
Alpha-Caryophyllene	0.95	-
Caryophyllene oxide	0.62	-
Methyl (10E)-10-heptadecen-8-ynoate	0.03	-
2-Myristynoyl pantetheine	0.06	-
Methyl 10-methylundecanoate	0.09	-
Methyl oleate	0.99	-
Alpha-Hydroxydodecanoic acid	0.04	-
9,12,15-octadecatrienoic acid	0.01	-
Beta- Pinene	-	1.18
D-Limonene	-	0.20
1.1-Dimethyl-2-octylcyclobutane	-	0.01
2-Nitrohept-2-en-1-ol	-	0.096
Z-α-Farnesene	-	1.24
Alpha-Humulene	-	5.92
Trans-Z-alpha-bisabolene	-	0.14
Trans-2-undecenoic acid	-	0.04
Doconexent	-	0.09
Benzyl oleate	-	0.146
3-trifluoroacetoxypentadecane	-	0.7
1-gala-1-ido-octose	-	0.12

$$*Relative\ Abundance = \frac{Area\ of\ each\ component}{Sum\ of\ areas} \times 100$$

III.3 Alpha-glucosidase inhibition assay

EO of Lebanese- cultivated *S. officinalis* induced a significant inhibition of α-glucosidase activity at almost all concentrations, except at 5%. As the concentration of *S. officinalis*' EO increased from 5% to 75%, the α-glucosidase inhibition increased in a concentration-dependent manner from 12.9% to 62.51% respectively.

As for the EO of *S. libanotica*, and in contrast with *S. officinalis'* EO, it did not inhibit the enzyme activity at low concentrations (5, 10, and 20%) but significantly did at higher concentrations (50 and 75%).

Both *S. officinalis* and *S. libanotica* induced the highest effect 62.51% and 63.15%, respectively, at the highest EO concentration used (75%). Moreover, at this concentration, the α-glucosidase inhibition was the closest and not statistically different from that reached with 20 mg/mL of acarbose used as positive control which inhibited the enzyme by 76.54% (Figure 1).

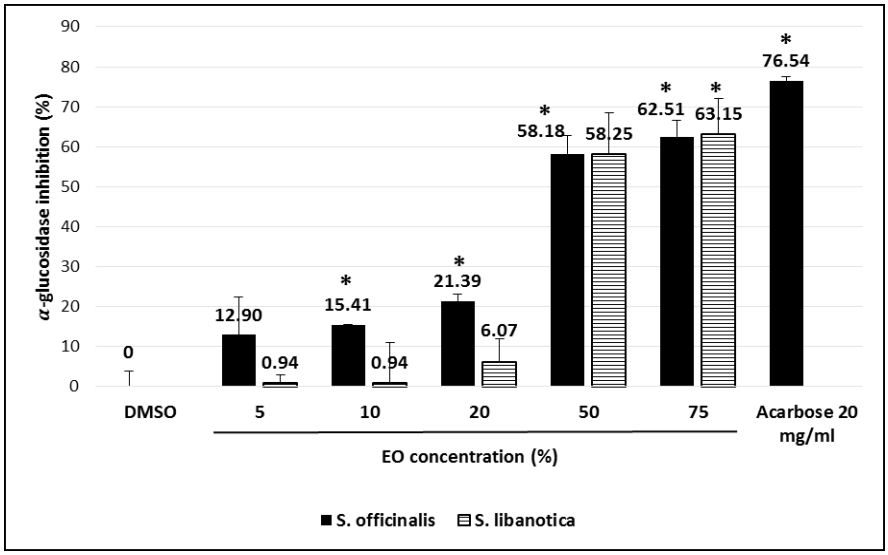

Figure 1: Alpha-glucosidase inhibition induced by *S. officinalis*' and *S. libanotica*'s EO.

The graph represents the effect of the negative control (DMSO), five concentrations of *S. officinalis*' and *S. libanotica*'s essential oil (EO), and the positive control (acarbose) at 20 mg/mL on α-glucosidase inhibition. Results are expressed as mean percentage ± SEM (n=3) and analyzed using non-parametric tests (Kruskal-Wallis followed by Mann Whitney). *: p≤ 0.05 compared to the negative control.

III.4 Albumin denaturation inhibition assay

Both *S. officinalis* and *S. libanotica* induced a significant inhibition of albumin denaturation at low concentrations (1%, 2.5% and 5%). Their effect decreased as the concentration of their EO increased, then it became zero.

The effect of *S. officinalis* was the highest (29.57%) at the lowest concentration used (1%), on the other hand, *S. libanotica* had the highest inhibitory effect (25.15%) at the concentration of 2.5%. Moreover, the highest effect induced by the two species wasn't statistically different from that of sodium diclofenac used at 250μg/mL, indicating their potent anti-inflammatory activity at these concentrations (Figure 2).

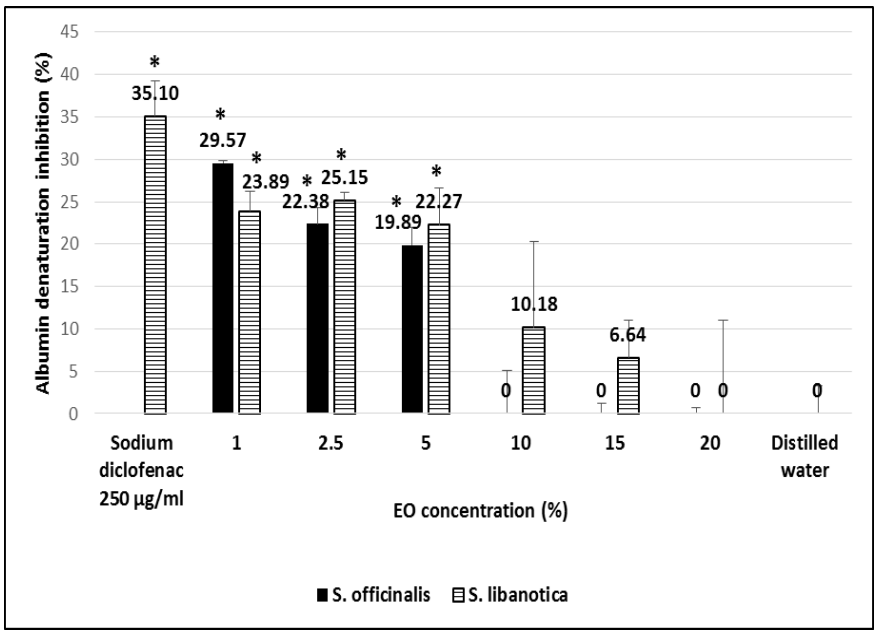

Figure 2: Albumin denaturation inhibition induced by the EO of *Salvia officinalis* and *S. libanotica*.

The graph represents the effect of the positive control (sodium diclofenac) at 250 µg/mL, six concentrations of *S. officinalis*' and *S. libanotica*'s essential oil (EO), and the negative control (distilled water) on the albumin denaturation inhibition assay performed at 57℃. Results are expressed as mean percentage ± SEM (n=3) and analyzed using non-parametric tests (Kruskal-Wallis followed by Mann Whitney). *: p≤ 0.05 compared to the negative control.

DISCUSSION

In the present study, EOs were extracted from both the Lebanese endemic *Salvia libanotica*, and Lebanese cultivated *Salvia officinalis'* leaves. Then, the *in-vitro* antidiabetic and anti-inflammatory activities of the two species were evaluated using the α-glucosidase and albumin denaturation inhibition assays, respectively.

First, hydro-distillation of air- dried leaves of Lebanese cultivated *S. officinalis* resulted in an EO yield of 1.75% w/w, higher than that obtained from Lebanese endemic *S. libanotica* (0.86% w/w) on a dry weight basis. This can be explained by the fact that, among different *Salvia* species, *S. officinalis* is known to have the highest essential oil yield (up to 3%)[12,13] Our results were close to the literature findings in the same conditions. Belhadj *et al* (2018) reported that *S. officinalis* leaves collected from Tunisia yielded 1.53% of EO.[14] Similarly, Lebanese *S. libanotica* has been reported to yield 0.7% of EO during pre-flowering stages, a value close to that obtained in our study (0.86%).[33]

The EO of *S. officinalis* and *S. libanotica* were then analyzed for their chemical composition. The GC/MS analysis of the EO of *S. officinalis* and that of *S. libanotica* revealed the presence of eighteen and twenty components, respectively. The predominant components (> 3%) of *S. officinalis* were alpha-thujone (38.56%), camphor (29.53%), eucalyptol (14.64%), alpha-humulene (5.92%), and camphene (5.53%). These components were also predominantly present in *S. officinalis*' EO from different countries, along with other components such as α- and β-pinene, viridiflorol, borneol, bornyl acetate, manool, and β-caryophyllene.[34] Furthermore, the main characteristic constituents of *S. officinalis* EO found in this study, are in accordance with the profile defined by the International Organization for Standardization (ISO 9909) which is α-thujone (18-43%), β-thujone (3-8.5%), camphor (4.5-24.5%), 1,8-cineole (5.5-13%), α-humulene (0-12%), α-pinene (1-6.5%), camphene (1.5-7%), limonene (0.5- 3%), linalool and bornyl acetate (2.5% maximum),[35] however the concentration of camphor and 1.8-cineole in our sample was slightly too high. As for *S. libanotica*, eucalyptol (64.27%), caryophyllene (10.59%), alpha-pinene (7.77%), camphor (5.28%) and 1R-alpha-pinene (3.82%) were predominantly present among the twenty components detected. This is in contrast with the study conducted by Bakkour *et al* (2011), also on Lebanese endemic *S. libanotica*, in which 34 components were characterized. Eucalyptol, camphor and α-pinene were detected, but in different proportions than our findings, along with other components such as β-pinene, camphene, β-myrcene and α-Terpineol.[36] The variation in the components' concentration between our results and the reported data can be attributed to many intrinsic and extrinsic factors including genetic background, locality altitude, season of harvesting, physiological stage, organ used for extraction, and environmental conditions such as temperature, day length, light intensity, water availability, soil salinity, etc.[34]

Salvia genus has been conventionally used for the treatment of various ailments since ancient times at various parts of the world. In recent years, many research studies have been conducted to document the traditional uses of *Salvia* species and to find new biological effects for this plant. These studies have revealed a wide range of pharmacological activities including anticancer, anti-inflammatory, anti-nociceptive, antioxidant, antimicrobial, antimutagenic, antidementia, hypoglycemic, and hypolipidemic, effect.[37] These activities vary between the cultivation regions according to the major components present, their types and their concentration due to environmental factors.[38] To our knowledge, no study, has evaluated the pharmacological activities of Lebanese cultivated *S. officinalis*, hence we were interested to study the antidiabetic and anti-inflammatory activities of the EO extracted from its leaves and compare these effects to those induced by Lebanese endemic *S. libanotica*.

The first investigated property was the antidiabetic effect evaluated by the α-glucosidase inhibition assay. Alpha-Glucosidase is a key digestive enzyme involved in the metabolism of carbohydrates: it catalyzes the hydrolysis of carbohydrates into monosaccharides, which makes this enzyme an important target for therapeutic control of diabetes and obesity. Alpha-glucosidase inhibitors (acarbose and miglitol) delay the digestion of carbohydrate and reduce the glucose absorption rate. However, this class of oral antidiabetic agents is associated with some side effects such as diarrhea, flatulence and abdominal pain.[39] Therefore, natural substances with less or no side effects are more and more researched.

Ours results showed a significant α-glucosidase inhibition at almost all concentrations of *S. officinalis* EO; starting with 12.90% inhibition at the lowest EO concentration (5%) and then increasing significantly and dose-dependently reaching a maximal inhibition of 62.51% at 75% EO concentration; the latter inhibition was close to that of the positive control acarbose (76.54%) used at 20mg/mL. These results reflect the ability of the EO of Lebanese cultivated *S. officinalis* to significantly inhibit the α-glucosidase enzyme at a starting dose of 10%.

Literature review revealed that there are no previous studies reporting the *in-vitro* α-glucosidase inhibitory effect of *S. officinalis'* EO. Few studies investigated the α-glucosidase inhibitory potential using *S. officinalis'* extracts. For example, *Salvia officinalis*' decoction induced 5-times more inhibition of α-glucosidase than did acarbose (EC50 = 71.2 µg/mL and 357.8 µg/mL, respectively).[40] This result is also consistent with a previous study reporting that the hydroethanolic extracts of *S. officinalis* had much stronger inhibitory effect

towards this enzyme than acarbose (IC_{50} = 69.7 µg/mL and 203.03 µg/mL respectively).[41] In contrast, the ethyl acetate fraction of *S. officinalis* revealed strong inhibitory effect (IC_{50}= 104.58 mg/mL) against α-glucosidase that remained lower than that of acarbose (IC_{50} of 42.52 mg/ mL).

Regarding the phytochemicals responsible of the antidiabetic activity, it is known that rosmarinic acid and other phenolic components (quercetin, caffeic acid …) of *Salvia officinalis* and triterpenes (such as Ursolic acid, Betulinic acid, Oleanolic acid and Corosolic acid) of *S. libanotica* have α-glucosidase inhibitory activities.[42,43]

The GC/MS analysis of *S. officinalis* EO evaluated in our laboratory showed that the EO' major components were α-thujone (38.56%), camphor (29.53%), eucalyptol (also known as 1,8-cineole, 14.64%), α-caryophyllene (5.92%) and camphene (5.53%). These results did not reveal the presence of phenolic compounds which may explain the high EO concentration required to inhibit the enzyme, and which suggest the implication of other components in inhibiting α-glucosidase. Indeed, α-thujone, present at 38.56% in *S. officinalis* oil, have been reported to possess an antidiabetic effect.[44]

Compared to the negative control, the EO of *S. libanotica* did not show any inhibitory potential for α-glucosidase at low concentrations (5, 10 and 20%). However, when the concentration of the EO substantially increased to 50%, the rate of inhibition increased (58.25%) and reached a maximum of 63.15% at the EO concentration of 75%. Moreover, at this concentration, the *α*-glucosidase inhibition was the closest to that reached with 20 mg/mL of acarbose (76.54%) and not statistically different from that reached with *S. officinalis* (62.51%).

The results obtained in our study are in contrast with those of Bassil *et al*. (2015) who showed that the administration of the aqueous extract of *S. libanotica* at different doses (50, 150 and 450 mg/Kg body weight respectively) on Spargue-Dawley rats that were fed a high-fat diet for six weeks had antidiabetic effect. In fact, they also showed that the intake of *S. libanotica* extract was associated with a significant decrease in fasting serum glucose as well as an increase in fasting serum insulin and liver glycogen content.[9]

*S. libanotica*s' EO is reported to contain Triterpenes such as Ursolic acid, Bleanolic acid… but our findings did not reveal the presence of those components which can partially explain why we didn't see a high antidiabetic effect which was not seen until the EO concentration reached 50%, a relatively high concentration possessing a great risk of toxicity. But this moderate effect seen at high concentration can be possibly explained by the presence of β-caryophyllene and *α*-terpineol both of which were shown to be α-glucosidase inhibitors. Regardless, their relative abundance wasn't high enough which explains why we needed a high EO concentration to inhibit the enzyme. Minor components found in our EO such as, Terpinolene and Linalool (0.49% and 0.08% respectively) were also found to be weak α-glucosidase inhibitors in other studies.[45,46] So, the effect seen could be in part due to the presence of major components such as β-caryophyllene and *α*-terpineol as well as some minor components such as Terpinolene and Linalool.

After evaluating the glucosidase inhibition activity, the anti-inflammatory potential of the two species was tested using the albumin denaturation inhibition assay, since inflammation has a central key in the pathogenesis of diabetes (138). In fact, in type I diabetes, the predominant theory is that the beta cell pancreatic islets are inflamed, called insulitis, through the course of the disease. As for type II diabetes, when the latter starts to develop, the body becomes less sensitive to insulin and the resulting insulin resistance also leads to inflammation. A vicious cycle can result, with more inflammation causing more insulin resistance and vice versa.[47] Thus, it becomes more and more clear that future research should focus on finding compounds with both antidiabetic and anti-inflammatory effects.

Compared to the negative control, *S. officinalis* EO showed significant dose-dependent decrease in albumin denaturation between 1 and 5%, then it became equal to zero starting at 10% oil concentration. A maximum inhibition rate (29.57%) was seen at the lowest EO's concentration used (1%) which was the closest to that of sodium diclofenac at 250 mg/mL (35.10%); and was not statistically different.

These results are in agreement with previous studies demonstrating the anti-inflammatory effect of the EO of *S. officinalis* collected from different countries. For instance, Albano *et al* (2012) revealed that the Portuguese *S. officinalis'* EO was able to inhibit 5-LOX, an enzyme involved in the biosynthesis of leukotrienes, a group of lipid mediators of inflammation derived from arachidonic acid.[48] De Melo *et al* (2012) demonstrated also that the oil of *S. officinalis* collected from Brazil significantly inhibited *in-vitro* and *in-vivo* leukocyte migration induced by casein and Carrageenan in rats, respectively.[49] Moreover, Tosun *et al* (2014) found that the EO of Albanian *S. officinalis* significantly inhibited the production of NO and nuclear factor kappa B (NF-κB) induced in response to the pro-inflammatory stimulus LPS in murine macrophages.[50]

The anti-inflammatory activity of our EO could be related to its constituents, namely □-thujones (38.56%), camphor (29.53%), 1,8-cineole (14.64%), and β- pinene (1.18%). In fact, it was shown that treatment with thujone downregulates the production of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin IL-1β, and IL-6 in metastatic animals.[51] Also, samples with high content in camphor were recommended as medicinal treatments due to their high

anti-inflammatory activity.[20,50] Moreover, 1,8-cineole (eucalyptol), a terpenoid oxide present in many plant essential oils such as *S. officinalis* EO, displayed an inhibitory effect on some types of experimental inflammation in rats, i.e. paw oedema induced by carrageenan and cotton pellet-induced granuloma.[52] Finally, β-pinene, present at 1.18% in our oil, has also been shown to exhibit anti-inflammatory effects.[53]

In comparison to our results obtained from the EO of *S. officinalis*, *S. libanotica* had shown, at the lowest concentration of EO used (1%), a lower anti-inflammatory effect (23.89%) than *S. officinalis* (29.57%). In addition, at 10% EO, *Salvia libanotica* still had a residual anti-inflammatory effect (10.18%).

Many previous studies already investigated the anti-inflammatory potential of various *S. libanotica*s' extracts, but to our knowledge this is the first study on its EO in this regard.

At low concentration we had a moderate effect which declined at high concentrations. This is in contrast with most studies investigating the anti-inflammatory potential of EOs but in agreement with some of them; for example, in their investigation on *Lippia sidoides'* EO, Monteiro *et al*. (2006) noticed that topical application of EO at lower doses (1 mg/ear) reduced more significantly ($P < 0.05$) the acute ear edema induced by 12-otetradecanoylphorbol 13- acetate (TPA) (45.93%) than higher doses (10 mg/ear, 35.26%). In addition, many well-known anti- inflammatory drugs are known to become pro-inflammatory at high concentrations, for example, Hydrocortisone can exert pro-inflammatory action at a high dose of 300mg.[54–56]

Collectively, our study demonstrated that, as the EO's concentration of *S. officinalis* and *S. libanotica* increased, the *α*-glucosidase inhibition increased, whereas the albumin denaturation inhibition decreased. The two species have however exhibited different potencies for these activities. This may be due to the quantitative and qualitative composition of their EO's chemical substances responsible of these pharmacological activities.

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