Phytochemical Compositions and Antidiabetic Potentials of *Salvia sclarea* L. Essential Oils

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Abstract: *Salvia sclarea* (SS) is characterized by its valuable essential oils (Eos) and potent biological activities. This study aimed at investigating the phytochemical composition of SS Eos collected in within the same week, from two different regions in Lebanon, Beirut (SS-Bt) and Taanayel (SS-Tl), utilizing GC-MS methods, and to explore their acute and subchronic antidiabetic potentials. Moreover, studying the phytochemical diversity of twenty SS Eos established on our work and literature descriptions in order to recognize the origin of the Lebanese active chemotype(s). The Eos have been obtained by hydro-distillation and identified via GC-MS analyses. Five chemotypes of SS Eos have been identified. The Lebanese Eos, SS-Bt and SS-Tl, studied here have shown evidence to belong to two different chemotypes 1 and 5, respectively. SS-Bt has shown to belong to chemotype 1, which is characterized by high linalool (LL) concentration (average 40.2%). On the other hand, SS-Tl has shown to belong to chemotype 5, which is characterized by high linalyl acetate (LA) concentration (average 50.4%). The acute and subchronic antidiabetic activities of these EOs have been monitored along with LL and LA, in order to find the most active chemotype. Chemotypes 1 (owned to high LL content), present at low altitude places of Lebanon and Poland, has shown significantly higher acute and subchronic antidiabetic activities than that of chemotype 5 (owned to high LA content). In conclusion, *Salvia sclarea* Eos have shown potential antidiabetic activities, and their Eos might be used in the future as a complementary or an alternative medicine in the management of diabetes and related complications.

Key words: *Salvia sclarea*, phytochemical composition, chemotype, essential oils, diabetes mellitus

1 INTRODUCTION

Salvia sclarea L. (Lamiaceae) (SS) is a biennial plant present in the Mediterranean countries and Africa till the Atlantic Ocean. It is also broadly cultivated in Europe and Asia for Eos extractive purposes¹⁾. In Lebanon, SS essential oils (EOs) originate widely in many regions, like Beirut (89 m above sea level) Beirut (SS-Bt), and Taanayel (882 m above sea level) (SS-Tl).

The aerial parts possess an intense aromatic scent owned to its valuable EOs. SS EO (SS-Eo) is distinguished by freshly floral-herbaceous scent, which is highly valuable for the fragrance and flavor manufacturing²⁾. Identification of EO is broadly utilized via GC-MS analyses, as it is a highly sensitive and selective technique for the detection and the identification of EO components¹⁾. Recent studies have shown that SS-EOs have some interesting biological activities. SS-Eos have been found to have anti-microbial, anti-inflammatory and antihyperalgesic activities³⁾.

Diabetes mellitus (DM) is a chronic endocrine metabolic disorder featured with hyperglycemia that is highly endemic worldwide⁴⁾. This disorder imposes a huge expense on the society, and the first step in its management is to control the blood glucose levels (BGL). The use of EO is one way of managing this disorder, and many plants and EOs have been tested and utilized in the prevention and management of DM⁴⁻⁶⁾. Other species of salvia (*S. fruticosa*) has shown to possess antidiabetic potentials⁷⁾. Till date, no previous study has reported EO profile of the Lebanese *S. sclarea*, and no detailed antidiabetic

Abbreviations: SS; *Salvia sclarea*, EO; essential oil, SS-Bt; *Salvia sclarea* collected from Beirut region, SS-Tl; *Salvia sclarea* collected from Taanayel region, SS-EO; *Salvia sclarea* essential oil, LL; Linalool, LA, linalyl acetate, BGL; Blood glucose level, CTRL; normal non-diabetic control, DIA CTRL; Vehicle treated diabetic control, GB; Glibenclamide 5 mg/Kg utilized as positive control; PCA; principle coordinate analysis.

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studies have been performed on SS-EO.

Therefore, the aim of the current study is to explore SS-EOs from two different regions in Lebanon (SS-Bt and SS-Tl) utilizing GC-MS methods and to explore their acute and subchronic antidiabetic potentials. Moreover, studying the phytochemical diversity of twenty SS-EOs established in our work and literature descriptions in order to recognize the origin of the active-chemotype(s) from Lebanon.

2 MATERIALS AND METHODS

2.1 Chemicals and Standards

All standards and chemicals used in this study have been obtained from Sigma-Aldrich (Germany). All solvents have been of analytical-grade and have been used without further purification.

2.2 Plant Material

Salvia sclarea aerial parts have been collected during the same week in April 2014 from Beirut (89 m above sea level), and Taanayel (882 m above sea level).

Plant authentication has been established on reference to "La Nouvelle Flore du Liban et de la Syrie"⁸⁾. Voucher specimens (PS-14-8 for SS-Bt and PS14-9 for SS-Tl) have been deposited in the faculty herbarium.

2.3 Extraction of EO

SS-Bt and SS-Tl EOs have been extracted for 3 h by hydro-distillation utilizing a Clevenger-apparatus, as illustrated before in the European-Pharmacopoeia⁹⁾.

2.4 GC-MS and Quantitative Analyses

SS-Bt and SS-Tl have been analyzed utilizing GC-apparatus associated with a mass-spectrometer selective detector (MSD) utilizing a GC-Agilent 6890 N Network (USA) and a network-detector Agilent 5975 for the MSD (USA). The GC has been also fitted with flame-ionization detector (FID). The GC-MS system has been also equipped with Wiley (Wiley, West-Sussex, England) and NIST (NIST 11.0, National Institute of Standards and Technology, Gaithersburg, MD) library-search databases. A DB-5 MS capillary column (30 m × 0.25 mm I.D., film thickness 0.10 µm) has been utilized as the stationary phase. Helium has been utilized as carrier-gas (0.7 mL/min). The temperature of the column has been initially adjusted to 35°C, has been increased gradually to 85°C (5°C/min rate), has been kept for 20 min at 85°C, elevated to 300°C (10°C/min rate), and conclusively has been kept at 300°C for 5 min. The sample has been diluted [1:100 (v/v)] and then, in splitless mode, has been injected at 250°C via auto-sampler. FID analysis has been done at 310°C. MS-spectra have been monitored at 70 eV with 310°C ion-source temperature and 320°C transfer-line temperature.

Most Eo components have been GC recognized by comparing their RI values with those reported before¹⁰⁾, with those of standard compounds purchased from Sigma-Aldrich (Germany), or with MS spectra provided with the Wiley and NIST libraries¹¹⁾. Without utilizing correction factors and as reported in Table 1, each compound relative-concentration has been determined dependant solely on the GC peak-area.

2.5 Statistical Analysis

Utilizing Origin Pro 2016 (USA), graphs of agglomerative and Heatmap hierarchical-clustering, and principle coordinate analysis (PCA) has been assembled that has been established on Euclidean-distance and Ward's-aggregation approach¹²⁾. The *in vivo* data is presented as means ± S.E.M. Statistical significance was tested by one-way analysis of variance with Fisher post-hoc test. Each point represents the mean ± S.E.M. for 7 animals. The *p*-value < 0.05 was designated as statistically-significant result.

2.6 Antidiabetic Activity

All animal studies were done according to the international animal welfare act and abiding by guidelines and approval of BAU-institutional review board (2017-A-0045-P-R-0218). Male (26-32 g) Swiss Webster mice (University animal house) were utilized in this study. The animals were housed in their cages one day before experimentation. The animals had free-access to standard-food and water with a 12 h alternation of dark and light cycles. Mice have been alloxinated (180 mg/Kg) in order to induce DM. All BGL levels have been measured via glucometers (Accu-chek, Germany). Animal having BGL levels ≥ 200 mg/dL have been recognized as diabetic and have been included in the antidiabetic studies. The experimental design and dosing have been summarized in Table. 4. All tested compounds have been administered orally utilizing gavages. The acute antidiabetic effect has been measured 0, 0.5, 2 and 6 h post-EO administration. While that of the subchronic antidiabetic activity the tested compounds have been administered every other day and have been measured for 8 days post-EO administration. All doses have been compared to vehicle (DMSO) diabetic control groups (DIA-CTRL), as reported before in literature⁶⁾. Glibenclamide 5 mg/Kg (GB) has been utilized in this study as a positive control¹³⁾.

3 RESULTS AND DISCUSSION

3.1 EOs Phytochemical Analyses

Phytochemical analyses of EOs *S. sclarea* as of Beirut (SS-Bt) the yield was 1.35% EO, whereas that from Taanayel (SS-Tl) the yield was 1.51% EO. Sixty-seven major-compounds have accounted for 86.80% of SS-Bt and

Table 1 Volatile compounds identified by the GC-MS method in the essential oil of *Salvia sclarea* (SS) from Beirut (SS-Bt) and Taanayel (SS-TI) .

Compound	Composition (peak area %)	
	SS-Bt	SS-TI
α -pinene	t*	0.09
β -pinene	0.09	0.09
camphene	0.09	t
myrcene	3.0	0.63
α -phellandrene	0.09	0.09
α -terpinene	t	0.09
p-cymene	0.09	0.45
limonene	0.08	0.18
(Z)- β -ocimene	0.09	0.09
(E)- β -ocimene	1.90	0.18
γ -terpinene	0.18	0.27
fenchone	0.09	t
terpinolene	0.05	t
linalool	38.07	10.75
endo-fenchol	0.18	t
pinocamphone	0.10	t
borneol	0.10	0.10
terpinen-4-ol	0.20	0.10
α -terpineol	13.40	6.45
nerol	2.07	1.00
pulegone	t	0.36
geraniol	5.67	4.40
linalyl acetate	1.00	35.28
neryl formate	0.10	t
bicycloelemene	0.18	t
neryl acetate	2.52	1.8
α -cubebene	0.10	0.10
geranyl acetate	4.86	3.45
α -copaene	1.1	0.9
β -bourbonene	0.18	t
β -elemene	0.27	t
α -cedrene	0.10	t
β -caryophyllene	1.00	2.26
isogermacrene	0.10	t
(E,E)- α -farnesene	t	0.27
β -ionone	0.18	t
germacrene D	2.00	10.60
β -selinene	t	0.18
bicyclogermacrene	0.27	1.18

Table 1 Continued.

Compound	Composition (peak area %)	
	SS-Bt	SS-TI
cubebol	0.10	t
δ -cadinene	0.27	0.36
α -calacorene	0.18	t
salviadienol	0.10	t
β -calacorene	0.10	t
spathulenol	1.45	0.18
caryophyllene oxide	1.34	0.50
salvial-4(14)en-1-one	0.36	t
humulene epoxide	0.10	0.10
torilenol	0.10	t
cedrol	0.18	t
12- <i>epi</i> -cedrol	0.18	t
humulene epoxide III	0.18	0.10
T-cadinol	0.18	t
β -eudesmol	0.45	0.45
α -eudesmol	0.27	0.27
eudesma-4(15),7-dien-1 β -ol	0.27	t
α -bisabolol	0.09	t
(<i>Z,Z</i>)-farnesol	0.10	0.20
(<i>E,E</i>)-farnesol	0.10	t
(<i>E</i>)-2,6-dimethyl-10-(p-tolyl)-undeca-2,6-diene	0.18	0.30
manoyl oxide	0.36	0.10
13- <i>epi</i> -manoyl oxide	0.18	0.10
manool	0.10	0.18
13- <i>epi</i> -manool	0.18	t
labda-7,14-dien-13-ol	0.10	t
Sclareol	0.10	1.18
Total Identified (%)	86.80	85.36

* t means less than 0.04%

85.36% of SS-TI have been identified. All 67 compounds have been identified and simultaneously quantified utilizing GC-MS methods (Table 1). The Eos consisted of twenty one oxygenated-monoterpenes (66.28% and 66.04% for SS-Bt and SS-TI, respectively), and thirteen monoterpene-hydrocarbons (7.92% and 7.68% for SS-Bt and SS-TI, respectively) (Table 2). Moreover, Eos also consisted of nineteen sesquiterpene-hydrocarbons (5.94% and 5.70% for SS-Bt and SS-TI, respectively), and eighteen oxygenated-sesquiterpenes (5.22% and 5.70% for SS-Bt and SS-TI, respectively) (Table 2). In order to understand the phytochemical diversity of *Salvia sclarea* species, a clustering-

hierarchical analysis (Fig. 1) has been conducted utilizing twenty aerial parts Eos from previous literature reported SS of Poland¹⁰, Iran¹⁴, Germany¹⁵, Italy I¹⁶, II³, III¹⁷ and IV², Turkey I¹⁸ and II¹⁹, Serbia²⁰, Bulgaria²¹, Austria²², China I, II and III²³, Brazil²⁴, Tajikistan²⁵, Russia¹⁷, and the EOs explored in the current study.

EO components have accounted for $\geq 4\%$ of at least one EO had been included in the statistical analysis and had been listed in Table 3, as previously reported in literature for other species of *Salvia*¹². Five chemotypes have been recognized, and the Lebanese EOs that have been studied in this study, have been found to belong to two different

Table 2 Terpenoids identified by the GC-MS method in the essential oil of *Salvia sclarea* (SS) from Beirut (SS-Bt) and Taanayel (SS-Tl) .

Terpenoids	Composition (Total peak areas %)	
	SS-Bt	SS-Tl
Monoterpene hydrocarbons	7.92	7.68
Oxygenated monoterpenes	66.28	66.04
Sesquiterpene hydrocarbons	5.94	5.70
Oxygenated sesquiterpenes	5.22	4.98
Others	1.44	0.96
Total Identified	86.80	85.36

chemotypes (Fig. 1). To verify the degree of phytochemical-variations, the major components (22) was subjected to clustering studies utilizing Principal coordinate analysis (PCA). Linalool (LL), alpha-terpeniol (AT), alpha-copaene (AC), and linalyl acetate (LA) accounted for 86.1% of the total variability. The dispersion of chemical variability analyzed by the PCA has suggested that SS populations were clearly differentiated to the 5 chemotypes (Fig. 2). Moreover, to determine the correlation between the different regions on the basis of their EO-composition, a Heatmap-cluster analysis has been performed. Heatmap-cluster analysis has shown the five main clusters (Fig. 3).

Chemotype 1 (cluster 1) has comprised the SS-Bt and the polish EOs. This cluster is characterized by relatively high linalool, alpha-terpeniol, geraniol and geranyl acetate proportions, with average of 40.2, 13.4, 6.0 and 5.2%, respectively. Chemotype 2 contains Iran, Germany and Italy (I) EOs, which has relatively intermediate proportions of alpha-terpeniol (av. 6.3%) compared to chemotypes 1. Chemotype 3 is restricted to Turkey, and it is characterized remarkably by high proportion of alpha-copaene (av. 3.9%). Comparatively to other chemotypes, the relative-proportion of alpha-copaene has been found to be significantly lower. Chemotype 4 is mainly present in Italy II, which has relatively the highest alpha-terpeniol proportion (47.4%). Relatively to other chemotypes, alpha-terpeniol has been found to be significantly lower. Chemotype 5 comprises the majority of the EOs studied, namely, Serbia, Bulgaria, Austria, Italy III and IV, China I, II and III, Brazil, Tajikistan, Russia, well as the SS-Tl from our study. Chemotype 5 is characterized by the highest linalyl acetate concentrations (av. 50.4%) relatively to other chemotypes. Moreover, chemotype 5 is also characterized by relatively lower levels of linalool and alpha-terpeniol, with average of 17.8% and 3.8%, respectively, when compared to chemotype 1.

Thus, GC-MS analyses and phytochemical diversity studies have shown that SS-Bt is rich in Linalool (LA) belonging to chemotype 1, and that SS-Tl is rich in linalyl acetate (LA) belonging to chemotype 5.

3.2 Acute and Subchronic Antidiabetic Activities

The acute (measured for 0, 0.5, 2 and 6 h), and subchronic (measured for 0, 1, 3, 5 and 8 days) antidiabetic activities for various doses of SS-Bt (50, 100 and 200 mg/Kg), along with various doses of its rich component LL (20, 40 and 80 mg/Kg) have been studied. Furthermore, the acute and subchronic antidiabetic activities for various doses of SS-Tl (50, 100 and 200 mg/Kg), along with various doses of its rich component LA (20, 40 and 80 mg/Kg) have been also monitored (Figs. 4 and 5). The SS-Bt at all doses has shown significantly ($p < 0.05$) the highest acute antidiabetic activity than those of SS-Tl. When compared to vehicle treated diabetic control, SS-Bt has shown acute reduction of 42.3%, 44.7% and 51.7% in BGL for doses 50, 100 and 200 mg/Kg, respectively, after 6h post-administration (Fig. 4). While acutely after 6h those of SS-Tl have shown 31.0%, 40.5% and 42.0% BGL reduction of, comparable same relative quantities, 50, 100 and 200 mg/Kg, respectively. In order to recognize the most active compounds responsible for SS-Bt and SS-Tl antidiabetic potentials, their most abounded compounds have been tested similarly in comparable doses to those present in the EO. LL (SS-Bt major EO component) at all doses has shown significantly ($p < 0.05$) higher acute antidiabetic activity than that of LA (SS-Tl major EO component). When compared to vehicle treated diabetic control, LL has shown acute reduction of 36.6%, 39.0% and 47.0% in BGL for doses 20, 40 and 80 mg/Kg, respectively. While acutely those of LA have shown 33.4%, 39.7% and 42.0% BGL reduction of, comparable same doses, 20, 40 and 80 mg/Kg, respectively. Acutely, LL has shown comparable results like SS-Bt, and LA has shown comparable results like SS-Tl. Thus, it could be concluded that LL and LA might be responsible for the acute antidiabetic activity of SS-BT and SS-Tl, respectively. Furthermore, SS-Bt at all doses has shown significantly ($p < 0.05$) the highest subchronic antidiabetic activity than those of SS-Tl. When compared to vehicle treated diabetic control, SS-Bt has shown after 8 day subchronically reduction of 44.1%, 50.2% and 52.0% in BGL for doses 50, 100 and 200 mg/Kg, respectively (Fig. 5). While after 8 days

Table 3 Literature survey of the chemical diversity and chemotypes of *S. sclarea* essential oils (Content of compounds in %).

Entry	Origin	myrcene	(E)- β -ocimene	γ -terpinene	linalool	endo-fenchol	terpinen-4-ol	α -terpineol	nerol	geraniol	linalyl acetate	neryl acetate	geranyl acetate	α -copaene	β -caryophyllene	germacrene D	bicyclogermacrene	δ -cadinene	spathulenol	caryophyllene oxide	β -eudesmol	manoyl oxide	Sclareol	Ref.
1	SS-Bt	3.0	1.9	0.2	38.1	0.2	0.2	13.4	2.1	5.7	1.0	2.5	4.9	1.1	1.0	2.0	0.3	0.3	1.5	1.3	0.5	0.4	0.1	This study
2	Poland	3.3	—	0.2	42.3	0.2	0.2	13.4	2.3	6.3	1.1	2.8	5.4	—	1.1	2.2	0.3	0.3	1.5	1.4	0.5	0.4	0.1	(Kuzma, 2009)
3	Iran	—	—	—	9.0	—	—	7.4	—	4.8	—	—	—	—	—	9.8	—	—	—	—	—	—	11.0	(Yousefzadi, 2007)
4	Germany	1.2	0.9	—	21.0	—	—	4.0	—	—	3.2	1.1	—	0.3	1.3	2.2	—	—	—	0.2	—	—	0.5	(Ernahrung, 2006)
5	Italy I	1.0	0.8	0.1	9.9	—	0.3	7.5	0.02	0.07	—	0.4	0.2	—	0.2	0.2	—	—	—	—	0.3	—	—	(Moretti, 1997)
6	Turkey I	—	—	—	—	—	—	—	—	—	—	—	—	4.0	5.1	1.3	0.5	—	11.4	24.1	—	—	11.5	(Yuce, 2014)
7	Turkey II	0.1	—	—	1.2	—	—	1.6	0.3	—	5.5	1.1	—	3.8	16.2	24.7	9.6	1.5	1.9	1.85	—	—	—	(ÖGÜTÇÜ, 2008)
8	Italy II	0.1	—	0.9	2.6	—	—	47.4	0.2	0.6	12.7	2.1	1.3	—	2.9	1.6	—	—	—	—	—	—	—	(Peana, 1999)
9	Serbia	1.0	0.7	—	18.2	—	—	5.0	0.3	—	52.8	0.5	—	0.6	1.8	0.8	0.2	0.1	0.1	0.3	—	—	0.1	(Džamic, 2008)
10	Bulgaria	0.5	0.2	—	20.8	—	0.04	2.6	0.4	—	56.9	0.7	1.2	0.9	3.4	5.1	1.4	0.2	0.2	0.2	—	—	0.2	(Hristova, 2013)
11	Austria	1.1	0.8	—	17.9	—	—	2.6	0.1	—	63.3	1.1	2.1	0.3	0.9	2.6	—	—	—	—	—	—	5.9	(Schmiderer, 2008)
12	Italy III	3.3	3.0	0.1	10.1	—	0.01	1.6	—	—	55.7	1.1	0.2	2.1	3.8	7.6	—	0.6	0.1	0.3	—	—	0.2	(Hudaib, 2001)
13	China I	0.6	—	—	28.1	—	—	5.1	0.9	2.2	49.8	1.6	—	—	0.8	0.3	—	—	0.3	0.8	0.3	—	—	(Cai, 2006)
14	China II	0.2	0.1	—	28.8	—	—	4.4	0.9	2.1	51.6	1.3	—	—	1.0	0.6	—	—	0.2	0.7	0.1	—	—	(Cai, 2006)
15	Brazil	1.2	—	—	28.8	—	—	5.1	—	—	60.1	—	—	—	—	—	—	—	—	—	—	—	—	(Andrade, 2016)
16	Italy IV	2.7	0.4	—	8.8	—	—	0.8	0.2	—	67.5	1.3	2.4	1.0	2.5	—	—	—	—	—	—	—	—	(Tognolini, 2006)
17	SS-TI	0.6	0.2	0.3	10.8	—	0.1	6.5	1.0	4.4	35.3	1.8	3.5	1.0	2.3	10.6	1.2	0.4	0.2	0.5	0.5	0.1	1.2	This study
18	Tajikistan	0.7	0.2	0.3	12.5	—	0.1	5.5	1.1	—	39.2	1.9	3.5	1.0	—	11.4	1.2	0.4	0.2	0.2	0.5	—	1.2	(Sharopov, 2012)
19	Russia	0.7	0.8	0.04	12.0	—	0.04	3.5	—	—	42.8	0.1	0.1	1.0	3.0	4.4	—	0.3	0.2	0.4	—	—	1.2	(Hudaib, 2001)
20	China III	0.5	0.2	—	17.0	—	—	3.2	0.6	1.4	29.5	1.0	—	—	0.6	0.5	—	—	0.1	0.5	0.2	—	—	(Cai, 2006)

Table 4 Antidiabetic experimental design protocol. Acute (0, 0.5, 2 and 6 hrs) and subchronic (1, 3, 5, 8 days) effect of SS-Bt, SS-Tl, LL and LA on blood glucose levels.

Groups	n	Tested Substance(s)	Description
I	7	Control	Normal mice: Vehicle [DMSO], PO*
II	7	Diabetic Control	Diabetic mice: Vehicle, PO
III	7	GB	Diabetic mice: glibenclamide (GB) 5 mg/kg, PO
IV	7	SS-Bt	Diabetic mice: SS-Bt EO 50 mg/kg, PO
V	7	SS-Bt	Diabetic mice: SS-Bt EO 100 mg/kg, PO
VI	7	SS-Bt	Diabetic mice: SS-Bt EO 200 mg/kg, PO
VIII	7	SS-Tl	Diabetic mice: SS-Tl EO 50 mg/kg, PO
IX	7	SS-Tl	Diabetic mice: SS-Tl EO 100 mg/kg, PO
X	7	SS-Tl	Diabetic mice: SS-Tl EO 200 mg/kg, PO
XI	7	LL	Diabetic mice: LL 20 mg/kg, PO
XII	7	LL	Diabetic mice: LL 40 mg/kg, PO
XIII	7	LL	Diabetic mice: LL 80 mg/kg, PO
XIV	7	LA	Diabetic mice: LA 20 mg/kg, PO
XV	7	LA	Diabetic mice: LA 40 mg/kg, PO
XVI	7	LA	Diabetic mice: LA 80 mg/kg, PO

* PO = Oral administration.

Fig. 1 Degree of dissimilarity of the chemical compositions of 20 *S. sclarea* Eos (Table. 3, Entries 1-20). Eos 1 and 17 were investigated in this study. The dendrogram was obtained by agglomerative hierarchical clustering based on Euclidian distances with *Ward's* aggregation method. For the composition, countries of origin, and literature source of each EO, see Table 3.

those of SS-Tl have shown 40.6%, 42.1% and 44.0% BGL reduction to 50, 100 and 200 mg/Kg, respectively. LL at all doses has shown significantly ($p < 0.05$) higher subchronic antidiabetic activity than those of LA. When compared to vehicle treated diabetic control, LL has shown subchronic

Fig. 2 Principle Coordinate analysis (PCA) performed on linalool (LL), alpha-terpeniol (AT), alpha-copaene (AC), and linalyl acetate (LA) for *S. sclarea* five chemotypes.

reduction of 40.4%, 45.4% and 47.4% in BGL for doses 20, 40 and 80 mg/Kg, respectively, 8 days post-administration. While subchronically after 8 days those of LA have shown 33.6%, 34.4% and 37.8% BGL reduction for doses 20, 40 and 80 mg/Kg, respectively (Fig. 5). The tested compounds have shown significant hypoglycemic effects when admin-

Fig. 3 Heatmap-Cluster based on metabolite profiles among the twenty *S. sclarea* populations. Colors in the heat map correlated to the metabolite %: blue for higher level; violet for medium levels and red for lower levels. (Numbers are assigned to essential oil compounds listed in Table 3).

istered to diabetic mice. These compounds when tested on normal non-diabetic mice only the highest concentration of SS-Bt and LL had significant hypoglycemic activity when compared to normal non-diabetic mice control (Fig. 6). Nevertheless, these hypoglycemic effects did not reach toxic levels. Therefore, we can conclude that the tested compound possessed good safety profile within the tested doses. Volatile oils amelioration of diabetes might be due to their potential antioxidant activity²⁶⁾ as they suppress glucose-induced oxidative stress²⁷⁾. Moreover, volatile oils alpha-glucosidase inhibition potentials might also contribute to their antidiabetic activity²⁸⁾. Subchronically, LL has shown comparable results like SS-Bt, and LA has shown comparable results like SS-Tl. Thus, it could be concluded that LL and LA might be responsible also for the subchronic antidiabetic activity of SS-BT and SS-Tl, respectively. In this study, it is the first time to describe the potential acute and subchronic antidiabetic activities of SS-Bt or SS-Tl EOs.

There is an increasing need of further making clinical

trials in order to fully understand *Salvia sclarea* antidiabetic mechanism of actions. The problem towards clinical application of *Salvia sclarea* might be due to several reasons. One of the problems might be the internal use of *Salvia sclarea* which might cause stomach lesion and ulcers; which might be solved by utilizing specially designed enteric coated capsules. The extrapolation of *Salvia sclarea* doses from animals to human-subjects might be also problematic in clinical trials. Another problem might be facing the *Salvia sclarea* clinical trials, the lack of funding organizations and infrastructure especially in developing countries; which might be resolved by encouraging non-governmental organizations in funding the promising clinical trials.

4 CONCLUSION

In this study, five chemotypes of *S. sclarea* EOs were identified by comparing twenty *S. sclarea* EOs established

Fig. 4 Acute antidiabetic effects of (A) SS-Bt, (B) SS-Tl, (C) Linalool (LL), and (D) Linalyl acetate (LA).

Fig. 5 Subchronic antidiabetic effects of (A) SS-Bt, (B) SS-Tl, (C) Linalool (LL), and (D) Linalyl acetate (LA).

Fig. 6 Acute effects on normal non-diabetic mice of (A) SS-Bt, (B) SS-Tl, (C) Linalool (LL), and (D) Linalyl acetate (LA).

on this work and literature descriptions. The two examined EO samples from two different regions of Lebanon, SS-Bt and SS-Tl, have shown to belong to different chemotypes, 1 and 5, respectively. Chemotypes 1 and 5 have been found to exhibit acute and subchronic antidiabetic activity due to the high percentages of LL and LA, respectively. Therefore, EOs from these two chemotypes might be used in the future as a complementary or as an alternative medicine in the management of DM, for further studies.

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Conflicts of interest

Authors declare no conflicts of interest.

Author Contribution Statement

KR and JH collected the samples and revised the manuscript. KR did the experimental and statistical parts and wrote the manuscript.

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