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IN VITRO MODULATION OF METABOLIC SYNDROME ENZYMES AND PROLIFERATION OF OBESITY RELATED-COLORECTAL CANCER CELL LINE PANEL BY *SALVIA* SPECIES FROM JORDAN

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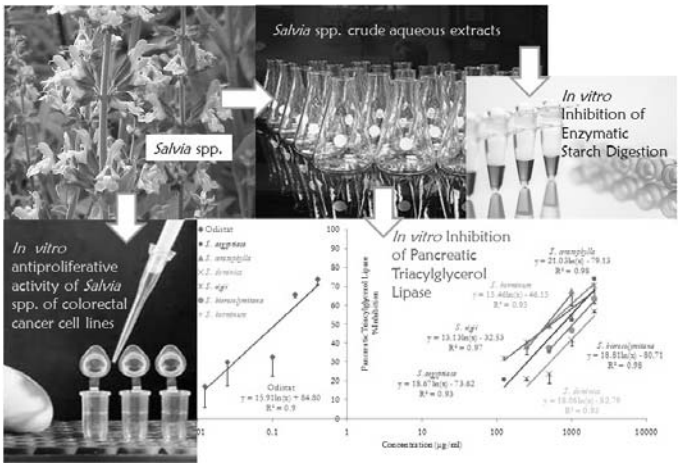
Pancreatic triacylglycerol lipase (PL), α-amylase and α-glucosidase are appealing pharmacological targets for the management of dyslipidemia, atherosclerosis, obesity and diabetes. Presently, *in vitro* screening for considerable inhibition of these digestive hydrolases by crude aqueous extracts (AEs) of eleven *Salvia* spp. grown and sold in Jordan (Lamiaceae) was undertaken. Similar to orlistat, eleven endemic species of *Salvia*s exerted pronounced dose dependent PL inhibition. PL- IC_{50} values (within a range of 0.14±0.02 – 1.51±0.17 mg/mL) in an ascending order were: *S. triloba* L. < *S. palaestina* Benth.< *S. ceratophylla* L. < *S. spinosa* Linn.< *S. eigii* Zohary < *S. aegyptiaca* L. < *S. syriaca* L. < *S. hierosolymitana* Boiss. < *S. lanigera* Poir.< *S. horminum* L.<*S. dominica* L. While 1,8-cineol was found inactive; PL- IC_{50} values (%) (V/V) of *Salvia* volatile oils' principles in an ascending order were: eugenol < α-thujone <α-terpinene. Comparable to acarbose, *Salvia* spp. AEs were identified as *in vitro* potent and efficacious dual inhibitors of α-amylase and α-glucosidase with IC_{50} values (within a range of 0.14±0.01 - 9.53±1.22 mg/mL) in an ascending order of: *S. horminum* < *S. lanigera* < *S. syriaca* < *S. triloba* < *S. spinosa* < *S. hierosolymitana* < *S. eigii* < *S. ceratophylla* < *S. palaestina* < *S. aegyptiaca* < *S. dominica*. Using SRB assay, except for *S. ceratophylla* and *S. eigii,* none of the tested *Salvia* spp. extracts for general cytotoxicity against a panel of colorectal cancer cell lines (HT29, HCT116, SW620 and Caco2) was found to possess cisplatin- or doxorobocin-like antiproliferative capacities in comparison to non induced basal incubations. Taken together, Jordan indigenous *Salvia* spp., modulating gastrointestinal carbohydrate and lipid digestion and absorption, maybe advocated as potential candidates for combinatorial obesity-diabetes (diabesity) prevention and phytotherapy.



INTRODUCTION

Globally and in Jordan, the escalating prevalence of obesity and diabetes is rather worrisome.[1-3] Obesity expedites the risk for developing diabetes, or a combination of cardiovascular disorders known as metabolic syndrome.[4] Combinatorial antiobesity-antidiabetes therapeutics from natural sources can stem from inhibition of digestive enzymes as pancreatic alpha-amylase, intestinal alpha-glucosidase and pancreatic triacylglycerol lipase.[5-7]

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Salvia is the predominant genus in the family Labiateae (Lamiaceae).[8-9] Collectively 19 indigenous species of *Salvia* are endemic to Jordan; [10-11] among which several *Salvia* species are closely linked to Jordanian traditional medicine of multiple ailments.[12-14] Amid the representatives of the genus *Salvia,* wildly grown and cultivated aromatic, edible and medicinally valuable species as well as ornamentals are present. *S. officinalis* L. was ascribed anti-inflammatory, antifungal and antinociceptive potentialities.[15-19] *S. triloba* essential oil and extract were recognized for their pronounced antibiofilm, antiadhesion and anti-MRSA effects.[20] Phytochemical screening using thin layer chromatography indicated the presence of terpenoids, flavonoids and coumarins in all examined extracts. Further details into terpenes' composition of *S. palaestina* Benth and *S. syriaca* L. from Jordanian origin were given elsewhere.[21-23] Impressively *Salvia* spp. extracts were reported to afford protective effects against oxidative and alkylating damage to DNA in human HCT15 and CO115 colon cells.[24] Additionally *S. fruticosa* leaves water extract was found protective against both H_2O_2-induced and intrinsic cellular DNA oxidation in human embryonic kidney 293 cells.[25] Moreover, *S. fruticosa* and *S. officinalis* induced pro-apoptotic antiproliferative efficacies in human colorectal HCT15 and CO115 cell lines.[26] Antioxidative capacities, relevant to phenolic contents of *Salvia* spp., were ascertained in multiple reports.[27-29]

In an attempt to add to the existing pool of pharmacological appraisals on *Salvia* species, the present study was designed to screen AEs of eleven indigenous *Salvia* species of Jordan for their antidiabesity activity via examining their inhibitory effectiveness of intestinal carbohydrate and lipid digestion and absorption enzymes. Furthermore, their potency was assessed in comparison to two standard drugs, namely acarbose and orlistat.[30-32] Their alleged cytotoxicity against a panel of colorectal carcinoma cell panel (HT29, HCT116, SW620 and Caco2) was further evaluated. Cisplatin and doxorobocin were the reference antineoplastic agents.

EXPERIMENTAL

Chemicals, biochemicals and instruments

Unless stated otherwise, all reagents and chemicals were procured from Sigma (Dorset, UK). Glucose GOD-PAP kit was obtained from BioLabo Reagents, France. In UV determinations; UV-VIS spectrophotometer from SpectroScan 80D (UK) was used. Sonicator (Bandelin Sonorex, Bandelin electronics, Germany) and rotary evaporator (Laborota 4000-efficient, Heidolph, Germany) were also used. Metformin and aspirin were procured from local suppliers.

Plant collection

S. aegyptiaca L., *S. ceratophylla* L., *S. dominica* L., *S. eigii* Zohary, *S. indica* L., *S. hierosolymitana* Boiss., *S. horminum* L., *S. lanigera* Poir. , *S. palaestina* Benth, *S. spinosa* Linn., *S. syriaca* L. and *S. triloba* L. (syn. *S. fruticosa* Mill.) *S. verbenaca* L. were collected from different regions in Jordan during early flowering period in 2012. *S. splendens* Sellow ex J.A. Schultes was purchased from a flowers exhibition in Amman. The plant material was identified by Professor Barakat Abu-Irmaileh, Faculty of Agriculture, The University of Jordan. Voucher specimens were deposited at the Faculty of Pharmacy, The University of Jordan. All plants were purified from extraneous material and dried at room temperature without direct exposure to sunshine.

Preparation of the *Salvia* spp. extracts

AEs were prepared by refluxing (without boiling) each 10 g of the dried coarsely powdered plant material with 100 mL tap water for 15 min. The overnight kept extracts were filtered twice through filter paper and the volume of the filtered solution was increased to 100 mL with tap water to obtain 10% (equivalent to 100 mg/1 mL) crude aqueous solutions.[33] Sonication of stock crude extract or testing concentrations was performed before implementation of investigations. For pancreatic lipase (PL) experimentation; water was evaporated under vacuum at 40 ^{o}C using a rotary evaporator. The solid residues were collected and stored in dry conditions until analysis. For cytotoxicity assay, each 10 g of the dried and coarsely powdered plant material was refluxed for 30 min using 70% ethanol, kept overnight, filtered and solvents were evaporated. 100 mg of extracts were dissolved in 10 mL DMSO (stock solution).

In vitro PL activity assay for *Salvia* spp. AEs, their volatile constituents and orlistat

Each of the tested AEs was initially dissolved in Tris-HCl buffer (2.5 mM (Promega, USA), pH 7.4 with 2.5 mM NaCl) to give five initial stock solutions with a concentration range 6.25 – 100 mg/mL (6.25, 12.5, 25, 50 and 100 mg/mL). Subsequently, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 125 – 2000 µg/mL (125, 250, 500, 1000 and 2000 µg/mL). Extracts were prepared according to the traditional indications of use, thus DMSO or any other organic solvent; even to the minimum concentration was avoided.[34] Also, eugenol, α-thujone, α-terpinene and 1,8-cineol (dissolved in DMSO)[21,35] were prepared into five initial stock solutions with an initial concentration range of 0.390 – 100 % (V/V) (0.390, 1.5625, 6.25, 25 and 100% (V/V)). Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range 0.00781 – 2% (V/V) (0.00781, 0.0312, 0.125, 0.5 and 2% (V/V)). Finally, orlistat, the reference drug (in DMSO; 1 mg/mL), was prepared in six different stock solutions with a concentration range of 0.625 - 20 µg/ mL.[36] Thereafter, 20 µL aliquot of each stock solution

was used in the reaction mixture to give a final concentration range of 0.0125 – 0.4 µg/ mL.

Spectrophotometric quantification of PL inhibition by *Salvia* spp. AEs and compounds

In vitro enzymatic PL activity was assayed according to Al-Hallaq et al.[37] Subsequent determinations were undertaken for the tested extracts or volatile oil compounds and orlistat in comparison to control evaluations, to calculate the concentration required for PL 50% inhibition (IC_{50}).

In vitro enzymatic starch digestion assay

In vitro enzymatic starch digestion was assayed with acarbose, as the reference drug.[38] The extent of polysaccharide breakdown into glucose was evaluated in a concentration range of plant aqueous extract 1, 5, 10, 12.5, 25, 50 and 100 mg/mL. The effects of acarbose at 1000 µg/mL concentration were evaluated as well. Control (tap water only) samples contained neither acarbose nor plant extract.

In vitro antiproliferative assay

Obesity related colorectal cell lines HT29, HCT116 and SW620 were generously provided by Dr Rick F. Thorne (University of Newcastle, Australia) and were cultured in high glucose DMEM containing 10% FCS (Bio Whittaker, Verviers, Pelgium). Caco2 cell line was a gift of Professor Yasser Bustanji, The University of Jordan, Faculty of Pharmacy. Caco2 cell line was cultured in RPMI 1640 containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (2 mM), Gentamicin (50 µg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL). The cytotoxicity measurements were determined using Sulforhodamine B (SRB) colorimetric assay for cytotoxicity screening and mechanism of reduction of cell viability as described previously.[39] Briefly cells were seeded at 5000/well onto flat bottomed 96 well culture plates and allowed to adhere overnight before the desired treatment into respective wells for the following 72 h in a CO_2 incubator with a humidified atmosphere of 5% CO_2 at 37 ℃. Hence afterwards, absorbance was read in a microplate reader at 570 nm. Human periodontal fibroblasts (PDL) are a primary cell culture for verification of selective cytotoxicity with the least antiproliferative IC_{50} value obtained. As positive controls, cisplatin and doxorobocin were recruited for comparison purposes.[39]

Statistical analysis

The values are presented as mean ± S.E.M. (Standard Error of the Mean) of 3-4 independent experiments. Statistical differences between control and different treatment groups were determined using Graphpad Prism one way analysis of variance (ANOVA) followed by Dunnett post test whenever appropriate (version 3.02 for windows; GraphPad Software, San Diego, CA, USA). Values were considered significantly different if $P<0.05$ and highly significantly different if $P<0.01$ and $P<0.001$.

RESULTS AND DISCUSSION

Salvias compose the largest genus in the Lamiaceae family. *Salvia* is derived from the Latin word salvare, "to heal," and for centuries many *Salvia*s have been valued for their medicinal and culinary qualities.[40] *S. triloba* and *S. hierosolymitana*, among other edible culinary herbs and food; have been recognized for their pronounced and beneficial medicinal properties.[35,41-42] Thus, these findings can provide useful indications for the promotion of these *Salvia* spp. as nutraceutical and functional food and/or for the extraction of bioactive health-promoting substances for pharmaceutical and food industries.

In vitro PL inhibitory effects of *Salvia* spp. AEs and selected volatile constituents

In the current study, the pancreatic triacylglycerol antilipase activity profiles of the AEs gradients of each of the eleven *Salvia* species and some of their volatile constituents (eugenol, α-thujone, and α-terpinene) are shown in Figs. 1A -C, respectively. Orlistat's PL-IC_{50} of 114.0 ± 4.0 ng/mL, equivalent to 0.2 ± 0.0 µM, is comparable to reported PL-IC_{50} values elsewhere (Table 1).[36] Similar to orlistat performance, a marked concentration dependent PL inhibition trend was obtained per tested extracts (the same figures) as well as their tested components, except for 1,8-cineol. PL-IC_{50} values obtained for a minimum of four separate determinations are also illustrated (Table 1).

Bioactive carnosic acid was identified as a new class of lipid absorption inhibitor from *S. triloba* with an anti-pancreatic lipase IC_{50} value of

12 µg/mL. Besides it could effectively reduce the gain of body weight and the accumulation of epididymal fat weight in high fat diet-fed mice after 14 days.[43] Thus, it was closely proven for its safe and effective clinical antihyperlipidemic effects in hypercholesterolemic and/or hypertriglyceridemic patients.[44] Mexican *S. microphylla* ethanol extract engendered mixed inhibition of porcine pancreatic lipase.[45] In Chinese medicine of atherosclerosis and cardiovascular diseases, *S. miltiorrhiza* Bunge was ascribed lipometabolism-regulatory efficacy for markedly lowering plasma triacylglycerol levels in high fat diet-induced hypertriglyceridemic mice.[46] Oral administration of *Salvia* spp. major constituent thujone (5 mg/Kg body weight) to diabetic rats normalized the cholesterol and triglyceride plasma levels.[47]

Fig. 1A

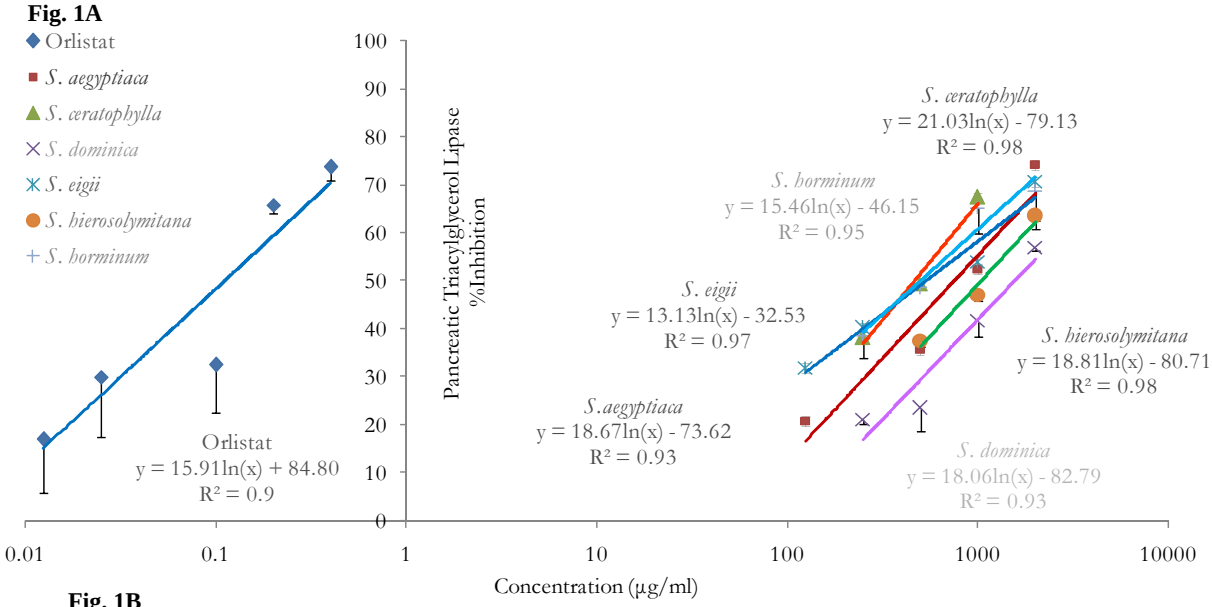

Fig. 1B

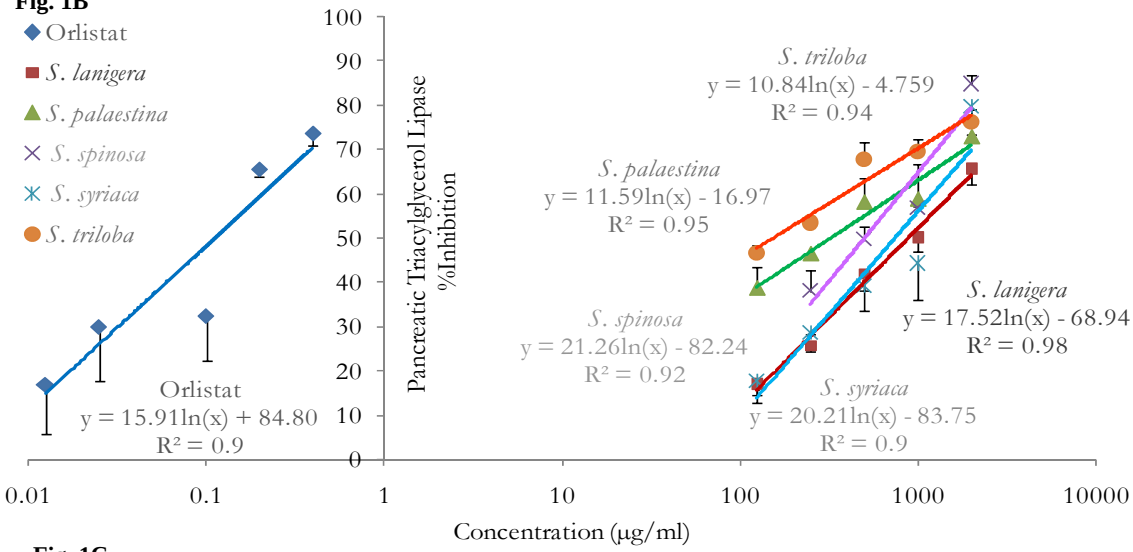

Fig. 1C

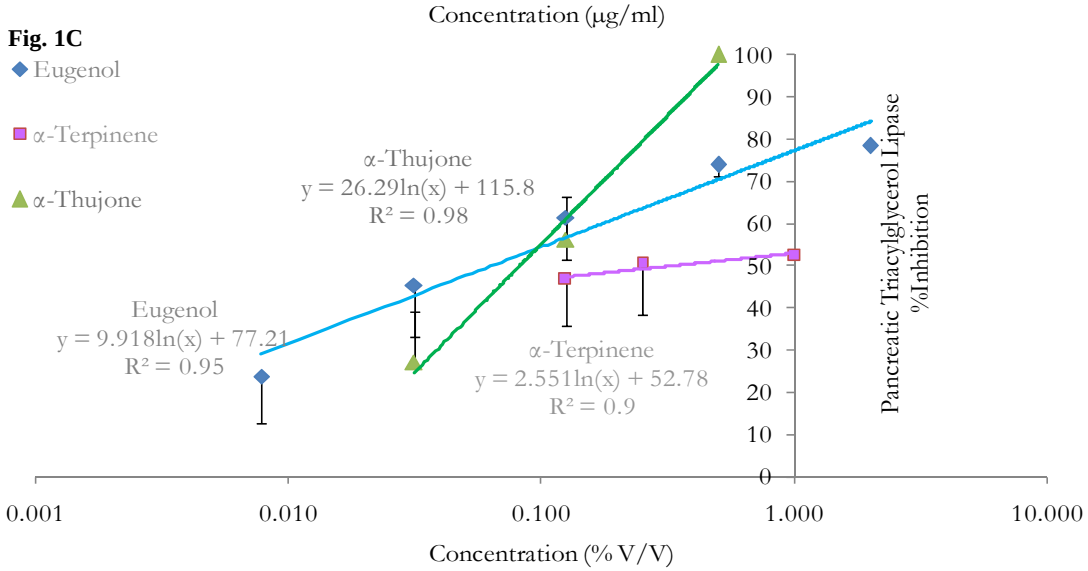

Fig. 1A-C – *In vitro* inhibitory effects of eleven *Salvia* spp. (AE) concentrations in µg/mL, *Salvia* essential oil principles and orlistat on Pancreatic Triacylglycerol Lipase Activity. **(A)** *S. aegyptiaca* L., *S. ceratophylla* L., *S. dominica* L., *S. eigii* Zohary, *S. hierosolymitana* Boiss., *S. horminum* L., **(B)** *S. lanigera* Poir. , *S. palaestina* Benth, *S. spinosa* Linn., *S. syriaca* L. and *S. fruticosa* Mill. (syn. *S. triloba* L.)). **(C)** Eugenol, α-terpinene and α-thujone. Results are mean ± SEM (n = 3 independent replicates).

Table 1

In vitro PL and enzymatic starch digestion IC_{50} values for increasing concentrations of eleven *Salvia* spp. AEs, volatile oil constituents, orlistat and acarbose. Results are mean ± SEM (n = 3independent replicates)

	IC_{50} (mg/mL)	
	Pancreatic Triacylglycerol Lipase	Enzymatic Starch Digestion
S. aegyptiaca	0.78±0.03	5.62±0.76
S. ceratophylla	0.45±0.07	4.43±0.72
S. dominica	1.51±0.17	9.53±1.22
S. eigii	0.51±0.05	4.36±0.32
S. hierosolymitana	1.00±0.09	3.56±0.16
S. horminum	0.53±0.02	0.14±0.01
S. lanigera	1.05±0.17	1.05±0.03
S. palaestina	0.33±0.03	5.14±0.76
S. spinosa	0.47±0.07	3.0±0.15
S. syriaca	0.84±0.10	2.03±0.18
S. triloba	0.14±0.02	2.59±0.14
Eugenol	0.06±0.01% V/V	-
α-Thujone	0.07±0.01 % V/V	-
α-Terpinene	0.33±0.07% V/V	-
1,8-Cineol	Inactive	-
Reference drug	Orlistat 0.114 ± 0.01 µg /mL	Acarbose 0.2± 0.02 µg /mL

In vitro inhibitory effects of *Salvia* spp. AEs on enzymatic starch digestion

In close likeness to the starch blocker acarbose, it is now believed that the inhibition of carbohydrate hydrolyzing enzymes in the digestive tract can significantly prolong the overall carbohydrate digestion time and decrease the postprandial hyperglycemia and hyperinsulinemia after a meal. Therefore, inhibitors of carbohydrate hydrolases can be useful therapeutic approaches in the management of obesity and type 2 diabetes mellitus and complications associated with the disease. Diverse studies were focused on the anti-amylase efficacies of volatile oils and compounds.[48-55] Flavonoids could often demonstrate the highest α-amylase inhibitory bioactivities, among phenolic phyto-constituents.[56-58]

With acarbose (0.1 mg/mL) as the reference drug, glucose liberation from starch was inhibited by 97.6% highly substantially ($p<0.001$, vs. drug-free control incubations, n=3, Fig. 2A-K). Furthermore, Fig. 2A-K demonstrate respectively that AEs concentrations 0.1-10 mg/mL of each of eleven *Salvia* species had highly substantial dose-related reductions in aldohexose release from culinary polymeric cornstarch ($p<0.05$ - $p<0.001$ vs. plant-free control determinations, n = 3). With IC_{50} (mg/mL) values enlisted in Table 1, the highly significant dose related ($p<0.001$) % decreases in enzymatic starch hydrolysis by each of eleven *Salvia* species dosage gradient (0.1-10 mg/mL) are summarized in Table 2.

Comparable outcomes were evidenced for other *Salvia* species. In OGTT of alloxan treated rats, *S. officinalis* significantly reduced postprandial blood glucose similar to acarbose, thus upregulation of Insulin and Glut-4 genes expression and inhibition of α-glucosidase activities were considered among the action modes that play a considerable role in hypoglycemic action of sage.[59] As for the Lebanese culinary herbs and traditional medicine of metabolic syndrome, *S. acetabulosa* methanol extracts exerted marked anti-α-amylase, and anti- α-glucosidase effects with respective IC_{50}s 91.2 µg/mL and 76.9 µg /mL, separately. The same extracts exhibited a strong inhibitory activity against ACE (angiotensin converting enzyme) with IC_{50} value of 52.7 µg/mL.[60] In addition to their phytotoxic and antifungal propensities, *S. moorcraftiana* Wall constituents were found to be effective inhibitors of α-glucosidase.[61] *S. verticillata* L. and *S. virgata* Jacq. ethanol extracts significantly and concentration dependently inhibited α-amylase activity using *in vitro* model, among other selected Iranian *Salvia* species (*S. hydrangea* DC., *S. hypoleuca* Benth., *S. officinalis* L., *S. reuterana* Boiss.).[27] Subsequently the bioactive flavone chrysoeriol of Iranian *S. virgata* ethanol preparation was found to exert a significant concentration dependent inhibition of α-amylase.[62] Besides, *S. miltiorrhiza* Bunge constituents were attributed with α-glucosidase and advanced glycation end product formation inhibitory activities.[63] Suggestively, given the anti-diabesity effectiveness added to proven safety of certain edible *Salvia* spp, successful *Salvia* spp. combinations maybe substantially incorporated into meal planning and formulations for diabetics.[64-67]

Fig. 2A

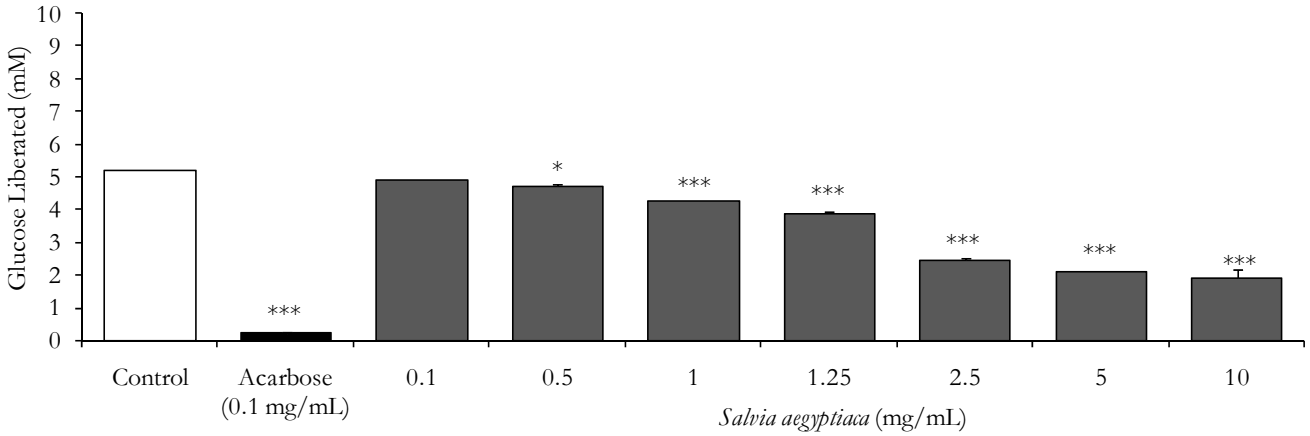

Fig. 2B

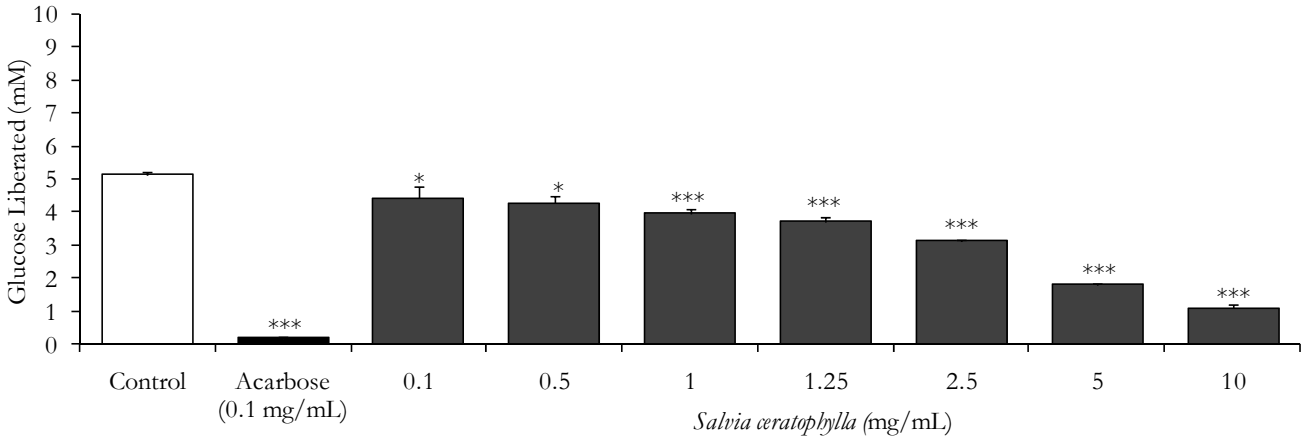

Fig. 2C

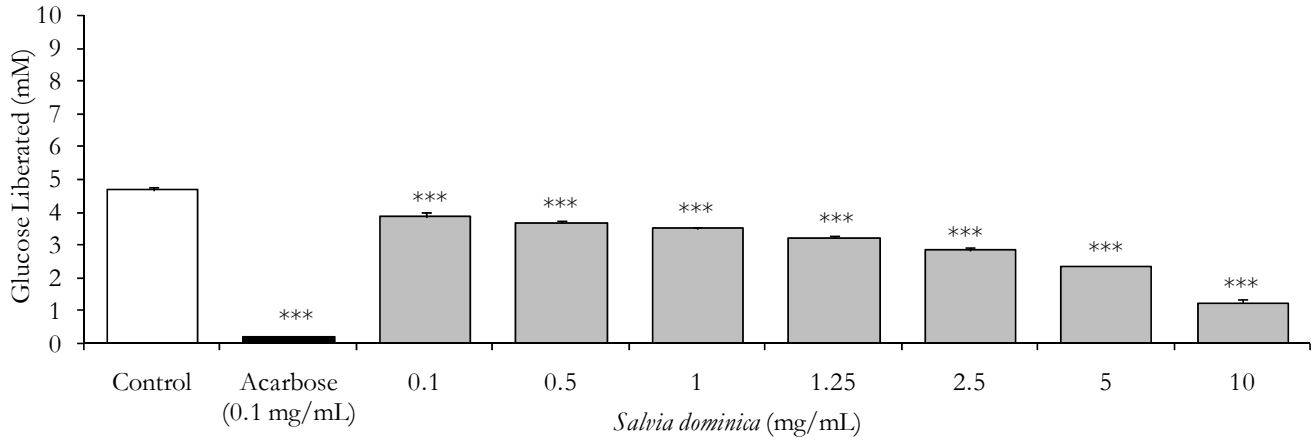

Fig. 2D

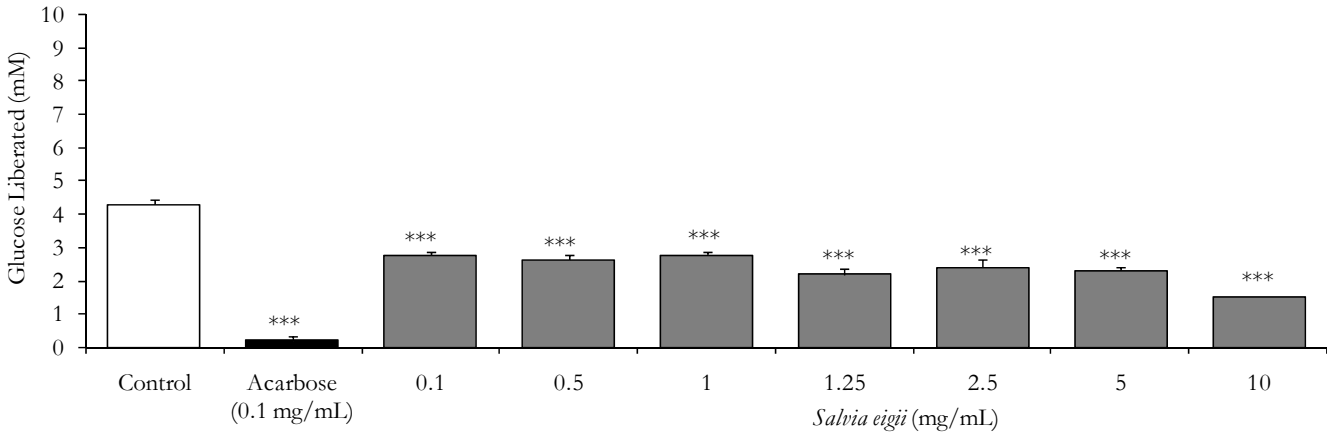

Fig. 2E

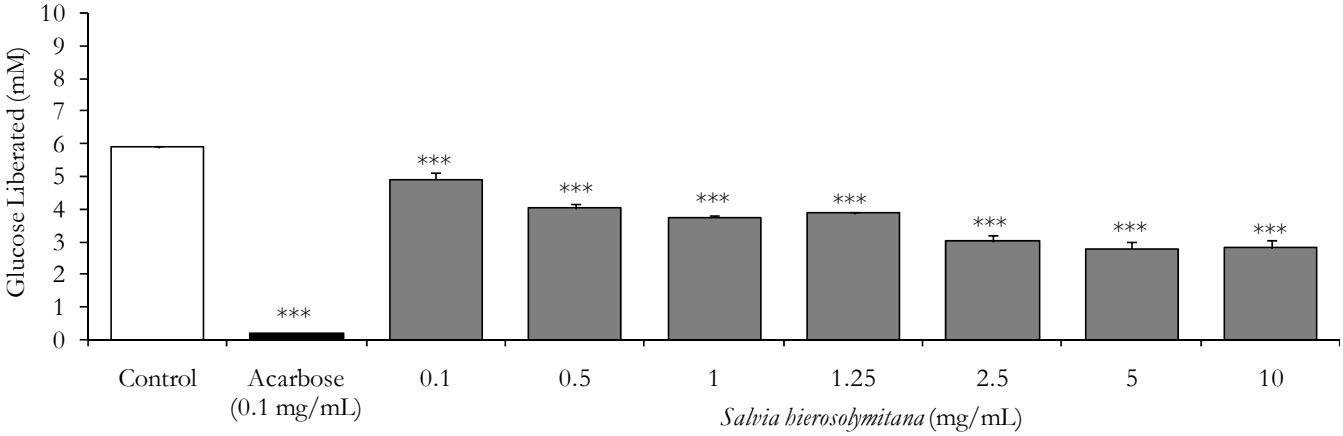

Fig. 2F

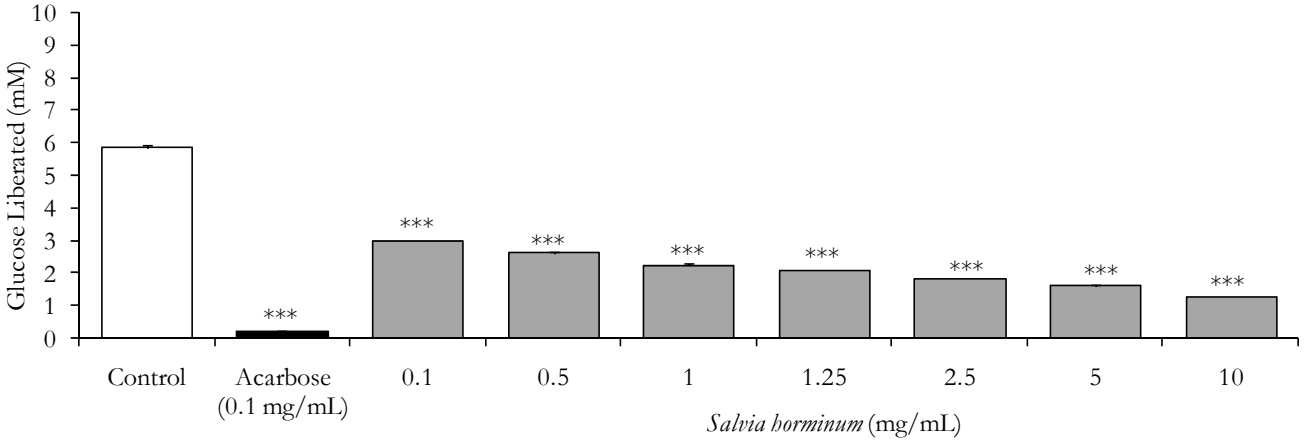

Fig. 2G

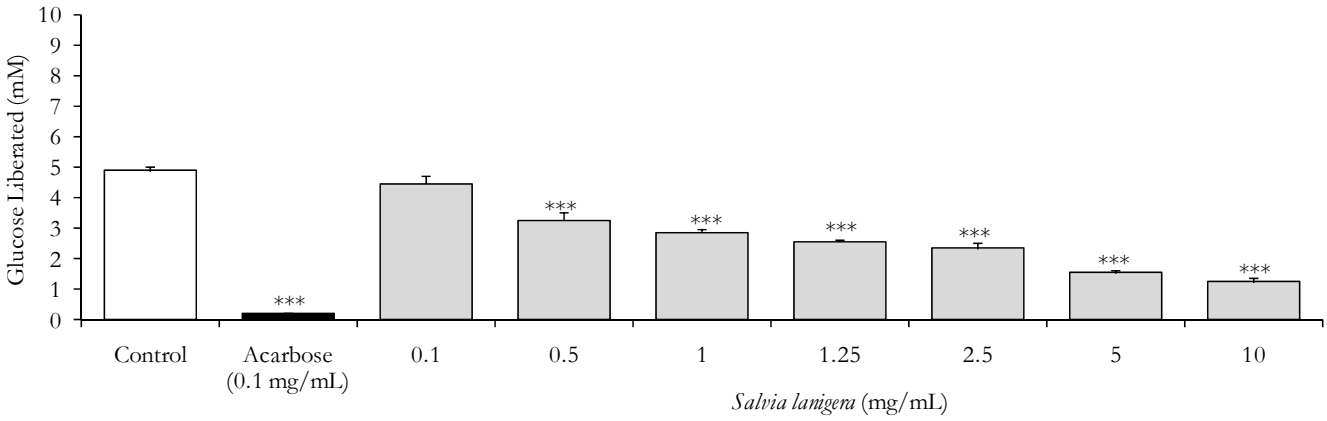

Fig. 2H

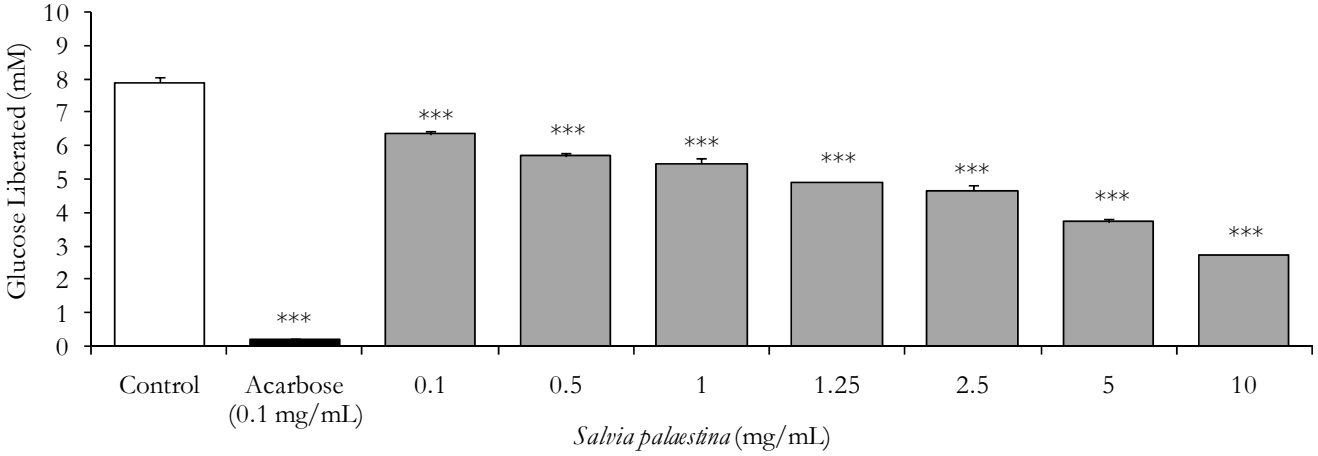

Fig. 2I

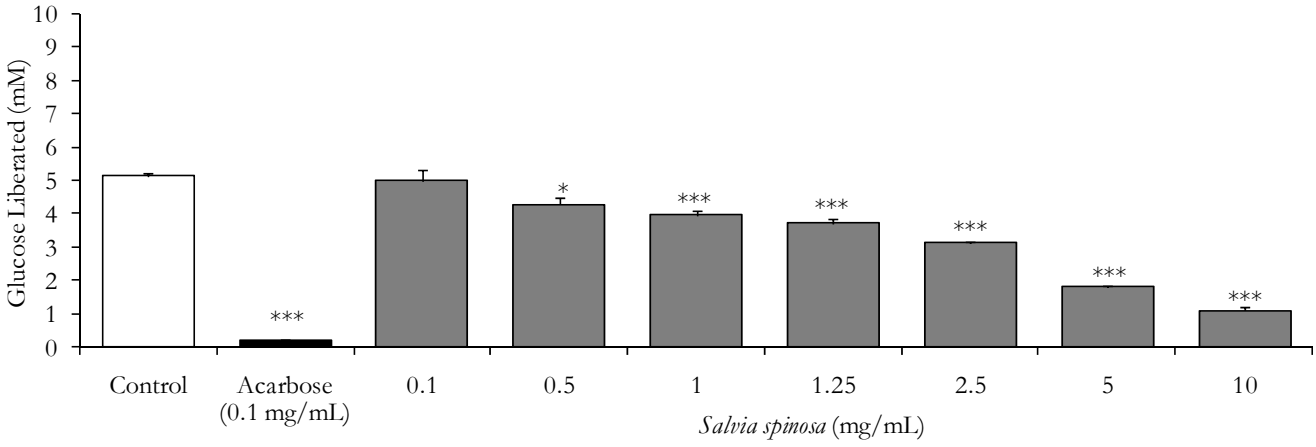

Fig. 2J

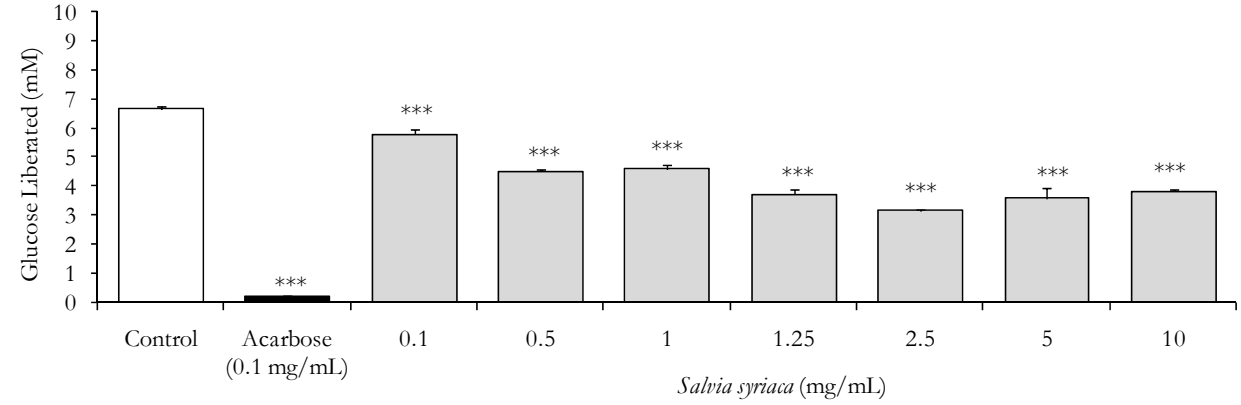

Fig. 2K

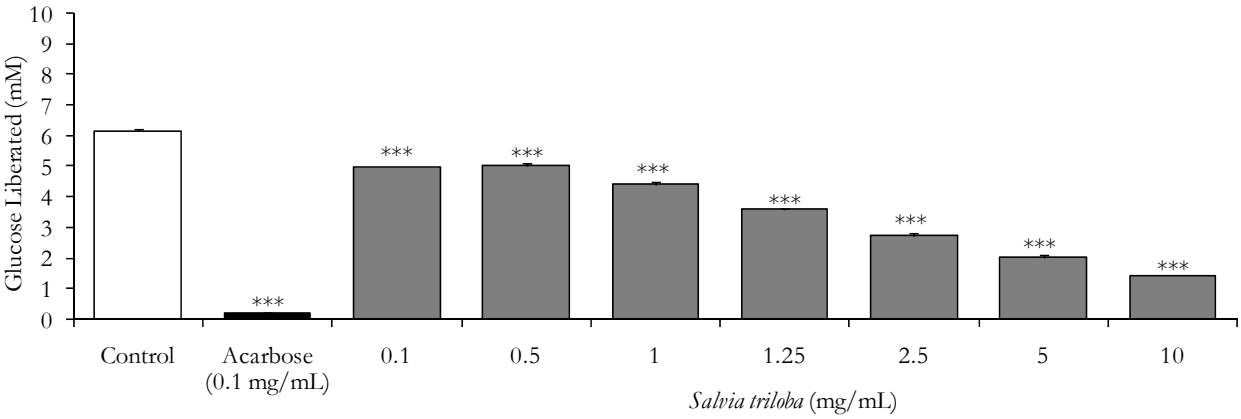

Fig. 2A-K – *In vitro* inhibitory effects of eleven indigenous *Salvia* spp. (AE) concentrations in mg/mL on enzymatic starch digestion **(A)** *S. aegyptiaca* L., **(B)** *S. ceratophylla* L., **(C)** *S. dominica* L., **(D)** *S. eigii* Zohary, **(E)** *S. hierosolymitana* Boiss., **(F)** *S. horminum* L., **(G)** *S. lanigera* Poir. , **(H)** *S. palaestina* Benth, **(I)** *S. spinosa* Linn., **(J)** *S. syriaca* L. and **(K)** *S. fruticosa* Mill. (syn. *S. triloba* L.)). Results are mean ± SEM (n = 3 independent replicates). **P<0.05* and ****P<0.001* compared to control (drug-free or plant-free) incubations, as determined by ANOVA followed by Dunnett post test.

Salvia spp. modulation of proliferative activity in obesity related colorectal cancer cell lines

Significant antiproliferative effectiveness of *S. dominica, S. triloba*, *S. horminum, S. syriaca* and *S. pinardi,* tested against a panel of cancer cell lines, was demonstrated with IC_{50}s < 30 μg/mL.[35, 68-69] *S. egyptiaca*, *S. syriaca*, *S. dominica, S. horminum* and *S. triloba* were reported also for their antiangiogenic properties, with selectivity against the endothelial cells proliferation, the key step in tumor angiogenesis.[70-71] Table 3 illustrates the antiproliferative efficacies of doxorobocin and cisplatin in all colorectal carcinomas tested. Presently, both S. *ceratophylla* and *S. eigii* were comparably cytotoxic against HCT116 72h incubations, considering NCI nominating of medicinal herb for downstream bioeffective principle(s)' purification if antiproliferative IC_{50} value was less than 30 μg/mL.[72] Exceptionally outstanding to the rest of tested *Salvia* spp., *S. ceratophylla* could exert antineoplastic efficacies in Caco2 incubations with an IC_{50} value of 9.2±0.5 μg/mL. Nevertheless, it lacked selective cytotoxicity in PDL fibroblasts wells (Table 3). Basically the rest of the tested *Salvia* spp. extracts were not impressively antiproliferative in any of the 4 colorectal carcinomas panel incubations despite their minor phytoprinciples α-terpinene evidenced effectiveness. Surprisingly *S. indica* was found noncytotoxic in relevance to any of colorectal carcinoma panel incubations, but it was cytotoxic against fibroblasts (the same table). Hypoglycemic metformin was observed to have growth inhibitory actions on colon cancer cells via activating AMPK, thus suggesting its particular value in attenuating the adverse effects of obesity on neoplasia.[73-75] So is acetylsalicylic acid being well known to exert antiprolifertaive effects and to induce apoptosis in colon carcinoma cell line HT29 [76-77] and in HCT116.[78] Nevertheless, Table 3 demonstrates that none of the tested metformin or aspirin concentrations (0.1-200 μg/mL) was principally antiproliferative/antineoplastic in any of the 4 colorectal carcinomas panel incubations.

Table 2

Effects of ascending concentrations of different *Salvia* spp. AEs (mg/mL) on %reduction of enzymatic starch digestion *in vitro*. Results expressed as % decrease in control values are mean ±SEM (n = 3 independent replicates). *$P<0.05$ and ***$P<0.001$ compared to control (drug-free or plant-free) incubations as determined by ANOVA followed by Dunnett post test

***Salvia* spp. AEs (mg/mL)**	**0.1**	**0.5**	**1.0**	**1.25**	**2.5**	**5**	**10**
S. aegyptiaca	6.4 ± 0.1	9.6 ± 1.1 *	18.5 ± 0.3 ***	25.5 ± 1.6 ***	53.2 ± 1.5 ***	59.6 ± 0.2 ***	63.1 ± 5.0 ***
S. ceratophylla	16.7 ± 4.6 *	17.0 ± 4.7 *	22.9 ± 2.7 ***	27.6 ± 2.3 ***	39.0 ± 5.4 ***	65.0 ± 1.1 ***	78.5 ± 2.4 ***
S. dominica	17.5 ± 3.0 ***	21.5 ± 2.0 ***	25.1 ± 0.9 ***	31.1 ± 3.4 ***	39.4 ± 2.1 ***	49.9 ± 0.9 ***	73.4 ± 2.1 ***
S. eigii	35.9 ± 0.9 ***	39.0± 2.3 ***	35.7 ± 2.5 ***	49.2 ± 3.8 ***	44.3 ± 1.6 ***	46.3 ± 2.1 ***	64.6 ± 0.7 ***
S. hierosolimitana	16.8 ± 3.3	31.7 ± 2.5 ***	36.7 ± 1.0 ***	36.3 ± 0.7 ***	49 ± 3.0 ***	52.8 ± 3.9 ***	52.1 ± 4.3 ***
S. horminum	49.3 ± 0.2 ***	55 ± 0.3 ***	61.5 ± 0.5 ***	64.4 ± 0.7 ***	68.2 ± 0.3 ***	72.4 ± 0.3 ***	78 ± 0.2 ***
S. lanigera	8.6 ± 3.2	30.8 ± 5.0 ***	41.5 ± 2.6 ***	47.6 ± 1.3 ***	52.3 ± 3.8 ***	68.6 ± 1.9 ***	74.7 ± 2.9 ***
S. palaestina	23.2 ± 0.8 ***	27.8 ± 0.7 ***	30.6± 2.2 ***	38.2 ± 0.1 ***	40.9 ± 1.8 ***	52.9 ± 0.9 ***	65.3 ± 0.4 ***
S. spinosa	4.4 ± 0.6	17.0 ± 4.7 *	23 ± 2.7 ***	28.3 ± 2.3 ***	40.0 ± 5.1 ***	67.0 ± 1.0 ***	79 ± 2.4 ***
S. syriaca	13.0 ± 2.1	32.3 ± 1.4 ***	30.9 ± 2.2 ***	44.2 ± 2.2 ***	52.6 ± 0.5 ***	46.2 ± 5.1 ***	42.4 ± 0.9 ***
S. triloba	18.8 ± 0.3 ***	18.5 ± 1.3 ***	28.4 ± 1.5 ***	41.5 ± 0.6 ***	55.8 ± 1.3 ***	67.1 ± 1.1 ***	76.6 ± 0.2 ***

Table 3

Lack of *in vitro* antiproliferative activity of *Salvia* spp. crude extracts on four colorectal cancer cell lines and PDL. Results, representing the IC_{50} (µg/mL), are mean ± SEM (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within treatment concentration range 0.1-200 µg/mL

Treatment	Cytotoxicity (as of %Control) (IC_{50} value: mean ± SEM µg/mL)				
	HT29 IC_{50}	**HCT116** IC_{50}	**SW620** IC_{50}	**Caco2** IC_{50}	**PDL** IC_{50}
S. ceratophylla	655.5±0.8	**28.02**±0.3	41.7±0.9	**9.2**±0.5	**0.01**±0.001
S. dominica	127.2±1.10	95.04±1.2	120.4±2.4	88.3±1.4	105.2±12.7
S. eigii	180.5±2.1	**30.33**±0.1	124±0.5	36.1±0.5	93.3±7.9
S. hierosolimitana	Non Toxic*	93.8±2.1	193.4±0.9	43.1±0.9	105.5±9.9
S. horminum	202.3±0.4	97±0.4	210±1.08	300.9±1.1	121.6±1.5
S. indica	Non Toxic*	128.4±0.78	172.6±1.1	77.4±1.7	**0.2**±0.05
S. splendens	61.8±0.5	58.7±0.2	129.8±1.3	41±1.3	55.8±1.7
S. palaestina AE	218±6.9	128.4±8.3	171.7±9.1	56.2±2.8	154.5±2.8
S. palaestina EE	115±4.7	89.9±5.2	123±4.3	63.8±3.1	97.4±2.3
S. spinosa	93.5±2.8	148.9±6.3	160.8±5.1	94.8±0.9	150±4.04
S. syriaca	215.4±5.1	163±1.06	157.2±3.6	71.3±0.8	134.7±5.5
S. triloba	109.5±2.3	116.9±2.8	106.3±5.8	131.1±4.7	133.6±9.2
S. verbenaca	139.1±1.7	51.7±1.4	126.9±2.1	51.7±2.1	78.8±1.8
α-Terpinene	**0.6**±0.04	**6.1**±0.07	202.3±12.3	**2.36±0.03**	4.9±0.05
1,8-Cineol	417.1±23.5	104±13.8	174.9±9.6	243.8±9.6	Non Toxic*
Doxorobocin	0.19±0.06	0.10±0.02	0.09±0.02	1.15±0.00	0.014±0.001
Cisplatin	1.13±0.01	3.5±0.08	2.6±0.02	4.3±0.05	1.01±0.02
Metformin	Non Toxic*	Non Toxic*	Non Toxic*	Non Toxic*	Non Toxic*
Aspirin	Non Toxic*	Non Toxic*	Non Toxic*	Non Toxic*	Non Toxic*

* Non toxic within the investigated concentration range (0.1 – 200 µg/mL). AE: aqueous extract. EE: ethanol extract

CONCLUSIONS

From the results of the study, it is inferred that the selected eleven indigenous *Salvia* species possess antidiabesity activity. Action mechanism of *Salvias*' enzyme inhibition maybe delineated. However, these herbs' propensities are warranted further *in vivo* models' testing and clinical trials for effective utilization as diabesity combinatorial phytotherapeutic and/or preventive agents.

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