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Phytochemical Profiling and Antidiabetic Activity of Algerian Medicinal Plants: In Vitro α-Amylase Inhibition and In Vivo Glucose-Lowering Effects

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Abstract

Various phytotherapeutic compounds with hypoglycemic properties offer a promising avenue to combat diabetes and its associated complications. This study was carried out to investigate the in vivo and in vitro antidiabetic activity and bioactive compounds of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur., and *Myrtus communis* L., which are used in Algerian traditional medicine. The in vivo antidiabetic assays of infusion extracts of the three plants at 100 and 500 mg/kg b.w, were performed using a hypoglycemic study in normal mice, an oral glucose tolerance test, and streptozotocin-induced diabetic mice. The α-amylase inhibitory test was used to determine the in vitro antidiabetic activity. Phytochemical composition and bioactive profiling were determined using HPLC analysis. The findings indicated a general agreement between in vivo and in vitro antidiabetic effects, rather than a direct quantitative correlation. In the in vivo antidiabetic activities, the infusion extract of *F. vesceritensis* displayed significantly ($P<0.05$) higher potency in reducing glycemia, glucose load–induced hyperglycemia, and in alleviating streptozotocin-induced diabetes, highlighting its potential as a source of bioactive compounds for therapeutic applications, with effects comparable to or higher than metformin under the tested conditions. *S. verbenaca* infusion extract showed a comparable blood glucose reduction to the reference drug. Similar findings were obtained in the α-amylase inhibitory test, where the infusion extract of *F. vesceritensis* showed strong inhibitory activity, while *S. verbenaca* also exhibited comparable inhibitory effects relative to the reference under the tested conditions. The phytochemical analysis revealed the presence of rutin and kaempferol in *F. vesceritensis* and *S. verbenaca*infusion extracts, and their absence in *M. communis* infusion extract, which may explain their observed bioactivity and supports their relevance as natural products with antidiabetic potential. However, further in vivo and clinical studies are required to confirm these findings and their translational relevance.

1. Introduction

Diabetes mellitus, characterized by chronic hyperglycemia, is a global health concern with an increasing prevalence across various populations [1]. Its impact on individuals, such as reduced treatment effectiveness and increased adverse effects and the burden it places on healthcare systems have spurred extensive research into novel approaches for its prevention and management. There is growing interest in exploring alternative, complementary, and natural sources for effective antidiabetic treatments [2]. The search for new and more sustainable treatments has led to the investigation of medicinal plants as sources of bioactive compounds with therapeutic potential. Globally, medicinal plants have served as an essential therapeutic resource, with over half of the global population relying on these plants, using extracts or active components as a traditional medicine to meet their fundamental healthcare needs [3]. These plants have shown potential in reducing blood glucose levels and mitigating the complications associated with diabetes [4, 5]. These plants have been traditionally used by local healers, and understanding their use through ethnopharmacological approaches is crucial to validate indigenous medical practices. Algeria possesses an important reservoir of medicinal plants owing to its rich biodiversity. In the southern regions of the nation, indigenous communities continue to depend on traditional healers for addressing various health concerns such as diabetes [6]. Among the medicinal plants used in Algerian herbal medicine for the treatment of diabetes are *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L.

Ferula vesceritensis Coss & Dur. is among the six species belonging to the *Ferula* genus in Algeria; this specie is abundant in south east of the country [7]. The aerial part of this plant is used in traditional medicine to treat headaches, fever, and throat infections [7]. In Algeria Sahara, the genus *Salvia* is represented by three species, including *Salvia verbenaca* subsp. clandestina (L.) Batt. [8]. Various traditional applications of this plant have been documented, such as reducing fever, easing digestive and spasmodic pain, and addressing liver disorders [9]. *Myrtus communis* L known as true myrtle, is a medicinal plant native to the Mediterranean area and belongs to the Myrtaceae family [10]. According to traditional medicine data, myrtle has been widely used for the treatment of various ailments, including pulmonary diseases, and viral and bacterial infections [11]. Ethnobotanical investigation indicates that these plants are widely used for reducing blood sugar levels [12–14]. However, to date, no scientific studies have comprehensively evaluated the bioactive compounds and their *in vitro* and *in vivo* antidiabetic activities. To our knowledge, this is the first comprehensive study integrating phytochemical profiling with *in vitro* and *in vivo* biological evaluation for these three medicinal plants used in Algerian traditional medicine. The aim of this study

was to evaluate antidiabetic activities of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L through *in vivo* and *in vitro* essays to verify the validity of their traditional use. Furthermore, a chromatographic phytochemical analysis was conducted to identify bioactive compounds potentially responsible for their antidiabetic activity.

2. Experimental

2.1. Plant Material

The aerial parts of *Salvia verbenaca* and *Ferula vesceritensis* Coss & Dur. were collected from El atteuf in Ghardaia region 32° 28' 60" north, 3° 40' 60" east (northern Algerian Sahara). *Myrtus communis* L. aerial parts were gathered from Cherchell station 36° 36' 27" north, 2° 11' 26" east, a coastal town west of Algiers.

The plants were identification by botanist, Prof. Saida Ouafı, according to Quezel and Santa [15]. Standard voucher specimens were conserved at the herbarium of Chemistry Department, University of Mentouri- Algeria (*Salvia verbenaca* : Sv15/137, *Ferula vesceritensis* : Fv03/10 and *Myrtus communis*: Mc32/61). The harvested plant aerial parts were air-dried until completely dehydrated at room temperature. Subsequently, the desiccated samples were finely powdered using an electric blender and stored in paper bags until their use.

2.2. Animals

Adult male albino mice, *Mus musculus*, weighing 20–25 g, were procured from the Pasteur Institute of Algeria. The animals were kept in a standard laboratory environment at a temperature of 24 ± 2˚C and a 12-hour light/dark cycle, with access to food and water provided *ad libitum.* The experimental procedures were approved by the ethical committee of the Algerian Association of Experimental Animal Sciences (88–08/1988) and conducted in adherence to the European Directive 2010/63/EU (EC, 2010) regarding the welfare of animals used for experimental and scientific purposes. The use of albino mice in the investigation of *in vivo* antidiabetic activity of plants infusion extracts was approved by the Ethical Committee of Animal Experimentation (CEEA) of University of Sciences and Technology Houari Boumediene (USTHB) with approved Ref N°: CEEA-USTHB-08-2023/11118, Algeria.

2.3. Plant extraction

The infusion extract of each plant sample was prepared using a traditional technique according to the method of Kraft and Hobbs [16], involving the addition of boiling distilled water/saline solution to the sample (1 g per 50 mL). After allowing it to steep at room temperature for 10 minutes, the mixture was filtered using Whatman grade No. 1 filter paper. Thereafter, the infusion extract underwent lyophilization and was reconstituted in methanol for chromatographic analysis [17].

2.4. *In vivo* antidiabetic assays

2.4.1. Grouping and dosing of animals

In the context of Streptozotocin-induced diabetes and hypoglycemic and oral glucose tests, mice were randomized into eight groups of five mice each. As a negative control, Group I was administered a solution containing 10 mL per kg b.w. of distilled water. Metformin was administered to Group II (the positive control) at 500 mg per kg b.w. *Salvia verbenaca* infusion extract was administered at 100 and 500 mg per kg b.w. to Groups III and IV, respectively. Similarly, the subjects in groups V and VI were administered infusion extract of *Ferula vesceritensis* Coss & Dur. at respective concentrations of 100 and 500 mg/kg body weight. In conclusion, infusion extract of *Myrtus communis L.* was administered to Groups VII and VIII at concentrations of 500 mg per kg b.w. and 100 mg per kg b.w., respectively.

2.4.2. Measurement of blood glucose level

Blood samples used for the blood glucose analysis were collected from the tail tip of each animal for all assessments. A blood glucometer (Accu Check Active, Roche, Germany) was employed to ascertain the blood glucose levels.

2.4.3. Hypoglycemic study in normal mice

The hypoglycemic effect of infusion extracts from three species was investigated in non-diabetic mice. The animals underwent a 16-hour overnight fasting period before the commencement of the experiment. A glucometer determined the initial fasting blood sugar levels the subsequent morning at 0 h. Subsequently, the animals in their respective groups were orally administered distilled water, the infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur., *Myrtus communis* L. (100 and 500 mg per kg b.w.) and the reference drug (Metformin 500 mg per kg b.w.). Blood glucose levels were subsequently measured at 1 hour, 3 hours, and 5 hours following the administration of the experimental medications in order to evaluate their hypoglycemic impact [18].

2.4.4. Oral glucose tolerance test

Oral glucose tolerance assay of infusion extracts of three plants was conducted in normal mice. After a 16-hour overnight fasting period, mice within their respective groups were orally administered various treatments: distilled water, infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur., *Myrtus communis* L. (100 and 500 mg per kg b.w.), and the reference drug (Metformin 500 mg per kg b.w.). Thirty minutes following this administration, the animals were orally administered a 40% glucose solution (4 g/kg). Blood glucose levels were measured prior to the treatments (representing baseline fasting glucose levels) and at 30 min, 60 min, 90 min, and 120 min after the injection of glucose [19]. The percentage decrease in the level of blood glucose following 120 minutes was calculated using the following:

% = ((Bgl $_{30\ min}$- Blgl $_{120\ min}$) / Bgl $_{30\ min}$) × 100 [20]

Bgl $_{30\ min}$

Blood glucose level after 30 min of glucose ingestion

Blgl $_{120\ min}$: Blood glucose level at t :120 min

2.4.5. Streptozotocin induced diabetes mice

After an overnight fasting period for the animals, blood glucose levels were measured before inducing diabetes. Diabetes was induced experimentally by administering freshly prepared streptozotocin (STZ) through intraperitoneal injection (45 mg per kg in 0.1 M sodium citrate buffer, pH 4.5) [21]. Thirty minutes after the injection, mice were given unrestricted delivery of food and water. Animals' diabetes confirmation was based on elevated blood glucose levels observed after 72 hours post-injection; only mice with fasting blood glucose levels exceeding 2 g/L were included in the study [22]. Following this, mice in their designated groups received different oral treatments: buffer solution, infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur., *Myrtus communis* L. at 1000 mg per kg b.w. and 500 mg per kg b.w. and the standard drug (Metformin 500 mg/kg b.w.). The levels of blood sugar were measured prior starting therapy (at 0 hours) and 2, 4, and 6 hours after administering the respective treatments [23, 24].

2.5. *In vitro* antidiabetic assay

2.5.1. Anti Alpha-amylase inhibitory test

The inhibitory activity of α-amylase was evaluated utilizing dinitrosalicylic acid [25]. Briefly, 100 µl of series of concentrations of infusion extract of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L (200 to 1200 µg/mL) and acarbose (as a reference) were mixed with 100 µl of (1 U/ml) α-amylase) and 200 µl of sodium phosphate buffer (pH 6.9; 20 mM,). After pre-incubating the samples for 10 min at 25°C, 200 µL of starch solution (1%) prepared in 20 mM sodium phosphate buffer (pH 6.9) was added. For ten minutes, the reaction mixtures were incubated at 25°C. To halt the reactions, 1 mL of dinitrosalicylic acid was added, then for ten minutes, the reaction mixtures were incubated at 25°C. Following the reduction of the reaction mixtures to ambient temperature, a 1:5 dilution with water was executed. Absorbance was subsequently assessed at 540 nm utilizing a CE2041 spectrophotometer (Cecil, Instruments, England). Each experiment was conducted in triplicate. The percentage of the enzyme inhibition was calculated using the following formula:

α-amylase inhibition (%) = $(Absorbance_{Control} - Absorbance_{Treatment})/(Absorbance_{Control}) \times 100$

2.6. HPLC analysis

The infusion extract from three species was analyzed using High-Performance Liquid Chromatography (HPLC) with an Agilent series 1100 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a UV detector and diode array detector (DAD, G1315B). The system was equipped with a quaternary rapid separation pump (G1376A). A Hypersil BDS-C18 column (250 × 4.6 mm, 5µm) served as the stationary phase. A linear gradient of acetic acid (0.2% in water) and acetonitrile over 30 minutes was utilized as the mobile phase, with an injection volume of 10 µL and a flow rate of 1.5 mL/min. Compound identification was based on comparing their retention times to standards.

2.7. Statistical analysis

The findings were reported as mean ± standard deviation (SD). In order to compare the samples, a one-way analysis of variance (ANOVA) was employed, followed by Tukey's multiple comparison test. The software Statistica version 6.0 was employed for this analysis. A statistically significant level of $P < 0.05$ was adopted to define significance.

3. Results and Discussion

3.1. Hypoglycemic study in normal mice

The objective of this investigation was to assess the impact of infusion extracts from *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L (at doses of 100 and 500 mg/kg body weight) on fasting blood sugar reduction at various times (1, 3 and 5 h). The results are presented in Table 1. The blood glucose level of control group remained unchanged throughout the entire experiment. The oral administration of the standard drug metformin (500 mg/kg b.w) decreased glucose level by 0.15 units after 5h of treatment. It was observed that the changes in mice glycaemia after oral administration of the infusion extracts were a dose-dependent trend for all three plants. The infusion extract of *Ferula vesceritensis* Coss & Dur. at dose of 500 mg/kg produced a statistically significant ($P < 0.05$) decrease of 0.20 units in blood glucose levels at all time intervals. This effect was comparable based on the observed reduction values to that of metformin. The infusion extract of *Salvia verbenaca* reduced the blood glucose levels of mice 5 hours after treatment by 0.09 and 0.12 units for the doses of 100 and 500 mg/kg, respectively, although these reductions were less pronounced compared to *Ferula vesceritensis* In contrast, *Myrtus communis* L did not produce statistically significant reductions in glycemia in normal mice at any time during the study, showing decreases of 0.06 and 0.07 units for the respective doses of 100 and 500 mg/kg.

These results suggest a relatively stronger glucose-lowering effect of *Ferula vesceritensis* and a moderate effect of *Salvia verbenaca*in normoglycemic mice.

Table 1
Effects of metformin and infusion extracts on the blood glucose levels of non-diabetic mice.

Treatment	Dose*	Time (h)			
		0	1	3	5
Control (saline)	---	0.98 ± 0.08	0.97 ± 0.07	0.95 ± 0.06	0.93 ± 0.07
Metformin	500	0.97 ± 0.07[a]	0.91 ± 0.06[a]	0.86 ± 0.06[a]	0.82 ± 0.05[a]
Salvia verbenaca	100	0.97 ± 0.10[a, b]	0.98 ± 0.08 [a, b]	0.93 ± 0.09 [a, b]	0.88 ± 0.10 [a, b]
	500	0.98 ± 0.07 [a, b]	0.95 ± 0.07 [a, b]	0.91 ± 0.07 [a, b]	0.86 ± 0.09 [a, b]
Ferula vesceritensis Coss & Dur.	100	0.94 ± 0.07 [a, b]	0.90 ± 0.07 [a, b]	0.84 ± 0.04 [a, b]	0.81 ± 0.07 [a, b]
	500	1.00 ± 0.08 [a, b]	0.93 ± 0.08 [a, b]	0.87 ± 0.07 [a, b]	0.80 ± 0.06 [a, b]
Myrtus communis L	100	0.99 ± 0.06 [a, b]	0.96 ± 0.06 [a, b]	0.94 ± 0.07 [a, b]	0.93 ± 0.06 [a, b]
	500	0.93 ± 0.10 [a, b]	0.91 ± 0.10 [a, b]	0.89 ± 0.10 [a, b]	0.86 ± 0.10 [a, b]

Dose* :(mg/kg b.w.); All values are expressed as mean ± SD, (n = 5); Tukey test: [a]$P < 0.05$ compared with control; [b]$P < 0.05$ compared with metformin.

3.2. Oral glucose tolerance test

The blood glucose levels and reduction percentages of the control, metformin (500 mg/kg b.w.), and the infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L. (100 and 500 mg/kg b.w.) at different times intervals (0, 30, 60, 90, and 120 min) are presented in Table 2. The results shown in Table 2 are also graphically illustrated in Fig. 1.

As illustrated in Table 2 and Fig. 1, peak blood glucose levels were observed in all groups compared to their baseline concentrations 30 minutes after the oral administration of an high glucose load (4 g/kg), which induced hyperglycemia in mice. After that, metformin (500 mg/kg b.w.) used as the reference medication significantly ($P < 0.05$) decreased blood glucose levels by 50.69%.

The infusion extract of *Ferula vesceritensis* Coss & Dur., when orally administered at 100 mg/kg b.w., reduced blood glucose levels ($P < 0.05$), although the percentage reduction was slightly lower than that of the reference drug (49.32%). Interestingly, at a concentration of 500 mg/kg b.w., the infusion extract showed a higher percentage reduction (59.09%) ($P < 0.05$) in glucose-induced hyperglycemia compared to metformin, representing the highest reduction among all tested groups under the present conditions. At 500 mg/kg b.w., the infusion extract of *Salvia verbenaca* significantly ($P < 0.05$) reduced blood glucose levels, achieving a reduction rate comparable to that of the reference drug (50.45%). Conversely, at 100 mg/kg b.w., the percentage decrease in blood glucose was slightly lower (44.74%) than that observed with metformin. Moreover, the infusion extract of *Myrtus communis* L. at both doses (100 mg/Kg and 500 mg/kg b.w.) did not produce significant changes in blood glucose levels compared to the control group, with reduction percentages of 18.84% and 24.41% for the respective doses.

These findings suggest that *Ferula vesceritensis* may exert a relatively stronger effect in reducing glucose-induced hyperglycemia under the present experimental conditions, while *Salvia verbenaca* shows a moderate effect.

Table 2. The blood sugar levels of mice subjected to a tolerance test for oral glucose compared with metformin and the infusion extracts.

Treatment	Dose*	Blood glucose level (g/L)					
		Time (min)					
		0	30	60	90	120	Reduction %
Control (saline)	---	1.05±0.03	2.08±0.04	1.95±0.04	1.88±0.03	1.79±0.01	13.94
Metformin	500	1.04±0.07	2.15±0.03	1.83±0.05	1.56±0.05	1.06±0.07	50.69[a]
Salvia verbenaca	100	1.03±0.07	2.19±0.03	1.78±0.04	1.60±0.03	1.21±0.03	44.74 [a, b]
	500	0.98±0.07	2.18±0.04	1.72±0.03	1.43±0.04	1.08±0.06	50.45 [a, b]
***Ferula vesceritensis* Coss & Dur.**	100	1.01±0.06	2.21±0.02	1.87±0.07	1.55±0.04	1.12±0.04	49.32 [a, b]
	500	1.01±0.07	2.20±0.01	1.72±0.03	1.48±0.03	0.90±0.09	59.09 [a, b]
Myrtus communis L	100	1.03±0.06	2.07±0.04	1.90±0.04	1.79±0.04	1.68±0.02	18.84 [a, b]
	500	1.07±0.03	2.13±0.03	1.96±0.01	1.78±0.03	1.62±0.02	23.94 [a, b]

Dose* :(mg/kg b.w); All values are expressed as Mean ± SD, (n = 5); Tukey test: [a]P <0.05 compared with control; [b]P < 0.05 compared with Metformin.

3.3. Streptozotocin induced diabetes mice

Administering the infusion of each sample orally to diabetic mice induced by streptozotocin at doses of 100 and 500 mg per kg resulted in a significant reduction in blood glucose levels compared with the diabetic control group. This reduction was observed in a dose-dependent trend. Table 3 represents the blood glucose levels in diabetic mice before and after 2, 4, and 6 hours treatments.

The infusion extract of *Ferula vesceritensis* Coss & Dur. at a concentration of 500 mg/kg produced a marked reduction in blood glucose levels at all time points post-treatment, with values reaching 0.80 g/L at 6 h, which was comparable to metformin. *Salvia verbenaca* infusion extract showed a similar trend to metformin, with final glucose levels of 0.81 g/L at 6 h for the 500 mg/kg dose. In contrast, *Myrtus communis* L. infusion extract did not produce a marked reduction in blood glucose levels at the administered doses across all time points compared to other treatments. These data suggest that *Ferula vesceritensis* exhibits a relatively stronger glucose-lowering effect under streptozotocin-induced diabetic conditions, while *Salvia verbenaca* shows a comparable but slightly less consistent effect.

Table 3. The impact of infusion extracts and metformin on blood glucose levels in mice with diabetes induced by streptozotocin.

Treatment	Dose*	Blood glucose level (g/L)			
		Time (hour)			
		0	2	4	6
Control (saline)	---	2.47± 0.05	2.43±0.05	2.40±0.04	2.36±0.04
Metformin	500	2.56±0.14[a]	1.78±0.08[a]	1.15±0.04[a]	0.88±0.09[a]
Salvia verbenaca	100	2.57±0.07 [a, b]	1.72±0.06 [a, b]	1.2±0.04 [a, b]	0.94±0.04 [a, b]
	500	2.51±0.08 [a, b]	1.85±0.08 [a, b]	1.25±0.07 [a, b]	0.81±0.05 [a, b]
Ferula vesceritensis **Coss & Dur.**	100	2.59±0.14 [a, b]	1.82±0.08 [a, b]	1.18±0.04 [a, b]	0.92±0.05 [a, b]
	500	2.53±0.07 [a, b]	1.47±0.15 [a, b]	1.23±0.09 [a, b]	0.80±0.05 [a, b]
Myrtus communis **L**	100	2.52±0.10 [a, b]	2.41±0.09 [a]	2.36±.09 [a]	2.28±0.06 [a]
	500	2.49±0.09 [a, b]	2.39±0.05 [a, b]	2.28±0.01 [a, b]	2.18±0.02 [a, b]

Dose*: (mg per kg b.w); All values are expressed as Mean ± SD, (n = 5); Tukey test: [a]P <0.05 compared with control; [b]P < 0.05 compared with Metformin.

3.4. *In vitro* antidiabetic assay

3.4.1. Anti alpha-amylase test

In the present study, all infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L. exhibited a concentration-dependent trend in α-amylase inhibitory activity, as illustrated in Table 4. *Ferula vesceritensis* Coss & Dur. infusion extract was found to have a significantly higher α-amylase inhibition, ranging from 51.32 to 77.38% at the concentration range of 200–1200 µg/mL. The infusion extract of *Salvia verbenaca* was able to inhibit the enzyme between 48.05% and 73.26% within the same concentration range. The inhibition achieved by these plants was comparable to that of the reference (acarbose), ranging from 46.10% to 73.05% at the same concentrations. However, the infusion extract of *Myrtus communis* L exhibited low inhibitory activity against α-amylase, as indicated by our findings. The IC_{50} values were derived from graphs plotting α-amylase inhibition against infusion concentrations. The highest inhibitory capacity was observed in *Ferula vesceritensis* Coss & Dur. infusion extract (IC_{50} = 158.40 µg/mL), followed by the infusion extract of *Salvia verbenaca* (IC_{50} = 298.40 µg/mL). Nevertheless, the infusion extract of M*yrtus communis* L. did not induce significant inhibition of the amylase enzyme (IC_{50} = 2101.65 µg/mL). Taken together, these results are in general agreement with the in vivo findings, although direct correlations between in vitro enzyme inhibition and in vivo hypoglycemic effects should be interpreted with caution.

Table 4. Amylase inhibitory activity of infusion extracts in comparison with acarbose

Extracts	α-amylase inhibitory percentage						IC_{50}
Concentration µg/mL	200	400	600	800	1000	1200	**µg/mL**
Acarbose	46,10±0.001	50,43±0.001	55,05±0.001	61,14±0.001	67,09±0.002	73,05±0.001	380.74
Salvia verbenaca	48,05±0.006	52,05±0.001	58,11±0.002	62,01±0.001	67,42±0.001	73,26±0.004	298.40[a]
Ferula vesceritensis	52,05±0.002	55,41±0.002	61,36±0.004	66,12±0.002	71,21±0.002	77,38±0.003	158.40[a]
***Myrtus communis* L**	12.55±0.002	16.01±0.004	20.34±0.004	24.02±0.002	29.22±0.001	33.00±0.001	2101.65[b]

All values are expressed as Mean ± SD, (n = 3); Tukey test: Values with different letters (a, b) were significantly different compared with Acarbose ($P < 0.05$).

3.5. HPLC analysis

The infusion extracts were analyzed for the presence of bioactive substances using high-performance liquid chromatography. The chromatograms used to detect phenolic compounds are depicted in Fig. 2.

Table 5 displays the identified phenolic compounds, as illustrated in Fig. 2.

Table 5. Retention times of phenolic compounds identified in infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L.

Extract	Peak	Retention Time (min)	Compounds
Salvia verbenaca	1	1.962	Ascorbic acid
	2	5.789	Resorcinol
	3	6.176	1.2-dihydroxybenzene
	4	7.037	Caffeic acid
	5	7.398	Acid di-nito-Salicylic
	6	7.817	4-Hydroxybenzaldehyde
	7	8.403	Orientin
	8	8.551	Rutin
	9	9.146	Quercetin-3-*O*-glycoside
	10	9.404	Luteolin 7-*O*-glucoside
	11	9.699	Salicylic acid
	12	10.269	Apigenin-7-glucoside
	13	10.799	Spiraeoside
	14	12.529	3,4,5 trimethoxy trans cinnamic acid
	15	13.692	Quercetin
	16	14.805	Kaempferol
Ferula vesceritensis	1	1.962	Ascorbic acid
	2	3.253	Gallic acid
	3	4.173	Protocatechuic acid
	4	7.719	vanillic acid
	5	8.551	Rutin
	6	9.118	Ferulic acid
	7	10.222	Rosmarinic acid
	8	10.963	Sinapic acid
	9	13.692	Quercetin
	10	14.805	Kaempferol
Myrtus communis L	1	3.253	Gallic acid
	2	5.376	Ellagic acid
	3	7.394	iso vannillic acid
	4	9.118	Ferulic acid
	5	11.982	p anisic acid
	6	12.752	Luteolin
	7	13.692	Quercetin
	8	17.168	Apigenin

The HPLC analysis revealed the presence of various phenolic compounds in *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L. infusion extracts in polyphenols (Fig. 3). *Salvia verbenaca* infusion contains 16 compounds, 7 of which are phenolic acids and two flavonols: quercetin and kaempferol. The presence of compounds in the heterosidic state includes C-glycoside: Orientin, and five O-glycosides: rutin, spiraeoside, luteolin 7-O-glucoside, Apigenin-7-glucoside, Quercetin-3-b-glycoside. Quercetin and kaempferol are identified as flavonols. The infusion extract of *Ferula vesceritensis* Coss & Dur. contains 6 phenolic acids and two flavonols: Quercetin and Kaempferol. Rutin is identified as O-glycoside. Ascorbic acid is present as an organic compound in the infusion extracts of both plants. There are five phenolic acids present in *Myrtus communis* L. infusion extract, along with two flavones (luteolin and apigenin). Quercetin is identified as a flavonol.

The development of agents for diabetes management that lack adverse effects remains a challenge within the healthcare system [26].

In this context, the present research investigated the antidiabetic activity, both *in vitro* and *in vivo*, of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur., and *Myrtus communis* L., identified in Algerian ethnobotanical studies for their traditional use in diabetes treatment. In all antidiabetic experiments, the infusion extract of the three plants exhibited a general dose-dependent trend in reduction of blood glucose levels. Hypoglycemic tests are used to assess peripheral insulin action [27, 28]. It was observed that the infusion extract of *Ferula vesceritensis* Coss & Dur. showed a stronger glucose-lowering trend across time points compared with metformin and infusion extracts of *Salvia verbenaca* and *Myrtus communis* L. The glucose tolerance test is commonly employed to evaluate the body's capacity for glucose metabolism by identifying alterations in post-prandial glucose levels. This test serves as a diagnostic tool for conditions such as diabetes mellitus or insulin resistance [29]. The infusion extract of *Ferula vesceritensis* showed a higher reduction in glucose levels, with values comparable to or slightly higher than metformin under the present conditions. The infusion extract of *Salvia verbenaca* significantly decreased blood glucose levels, achieving a reduction rate similar to that of the reference drug. Similar results were obtained in streptozotocin-induced diabetes assay, regarded as an excellent experimental paradigm for evaluating the effectiveness of hypoglycemic drugs [30]. Streptozotocin, known for its cytotoxic effects, induces the demise of pancreatic β-cells through DNA alkylation, resulting in fewer active cells and consequently diminishing insulin synthesis and release [31]. The findings of this work suggest that *Ferula vesceritensis* may exhibit effects comparable to oral antihyperglycemic agents, leading to a reduction in blood glucose levels in normoglycemic animals [32]. *Salvia verbenaca* may act through mechanisms similar to metformin, based on the observed experimental trends [33]. In contrast, the infusion extract of *Myrtus communis* L, did not lead to a reduction in blood glucose levels in all in *vivo* experiments. The integration of *in vitro* screening methods with *in vivo* assessments offers a comprehensive approach to evaluating the efficacy of these medicinal plants. The α-amylase inhibitory test revealed that the infusion extract of *Ferula vesceritensis* exhibited higher inhibitory activity, followed by *Salvia verbenaca* infusion extract, and this inhibitory effect was comparable to that of acarbose. Unlike the infusion extract of *Myrtus communis* L., it did not show effectiveness. The results acquired from the in vivo experiments are generally consistent with the observations from the in vitro study, although direct correlations should be interpreted with caution. The main mechanism of action of the hypoglycemic effect of medicinal plants may involve the stimulation of insulin release by β cells or the inhibition of glucose absorption from the intestine or a combination of both [5, 34]. Phenolic constituents of plants can be considered as potential agents for diabetes management [26, 35]. The phytochemical investigation indicated the presence of rutin and kaempferol in *Ferula vesceritensis* and *Salvia verbenaca* infusion extracts, which have been reported to possess antidiabetic-related activities [36, 37]. These compounds may contribute to the observed hypoglycemic effects. Apart from these compounds, protocatechuic acid also exhibits antidiabetic-related activity [38, 39], and its presence in *Ferula vesceritensis* may partly explain its relatively stronger activity. Conversely, the phytochemical analysis of *Myrtus communis* L. suggests a lower abundance of compounds commonly associated with antidiabetic activity, which may explain its limited effect in the present study.

4. Conclusion

This study is the first to specifically evaluate the in vitro and in vivo antidiabetic properties of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur., and *Myrtus communis* L., which are commonly used in Algerian traditional medicine for diabetes

management. Based on the obtained results, it can be suggested that the infusion extracts of *Salvia verbenaca* and *Ferula vesceritensis* exhibit significant hypoglycemic activity, which may be associated with their phytochemical composition, particularly rutin, kaempferol, and protocatechuic acid. These findings highlight the potential relevance of these plants as natural sources of bioactive compounds for diabetes management and support their ethnopharmacological use, although further studies are required to confirm their efficacy and safety and to support the development of plant-derived antidiabetic compounds.

Declarations

Ethical Approval The experimental procedures were approved by the ethical committee of the Algerian Association of Experimental Animal Sciences (88–08/1988) and conducted in adherence to the European Directive 2010/63/EU (EC, 2010) regarding the welfare of animals used for experimental and scientific purposes.

Competing interests / COI statement: Not applicable.

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Authors' contributions:

Lilya Harchaoui: Methodology, Conceptualization, Software, Data curation, Writing – review & editing. **Saida Ouafi:** Visualization, Validation. **Wafa Zahnit**: Conceptualization, Writing – review & editing. **Majid Sharifi-Rad:** Writing – review & editing. **Mohammed Messaoudi:** Writing – review & editing.

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Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

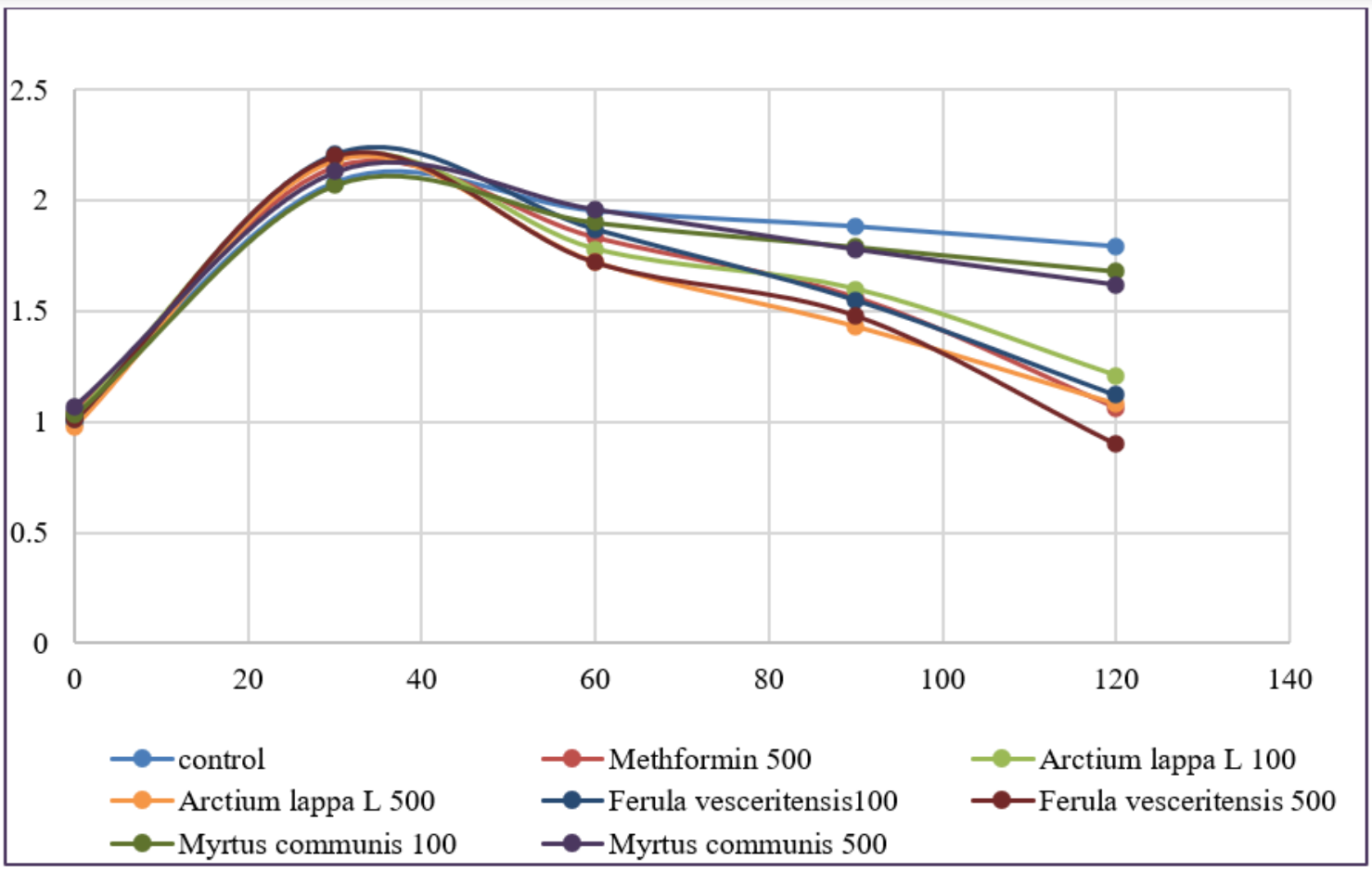


Figure 1

Oral glucose tolerance test (OGTT) in mice

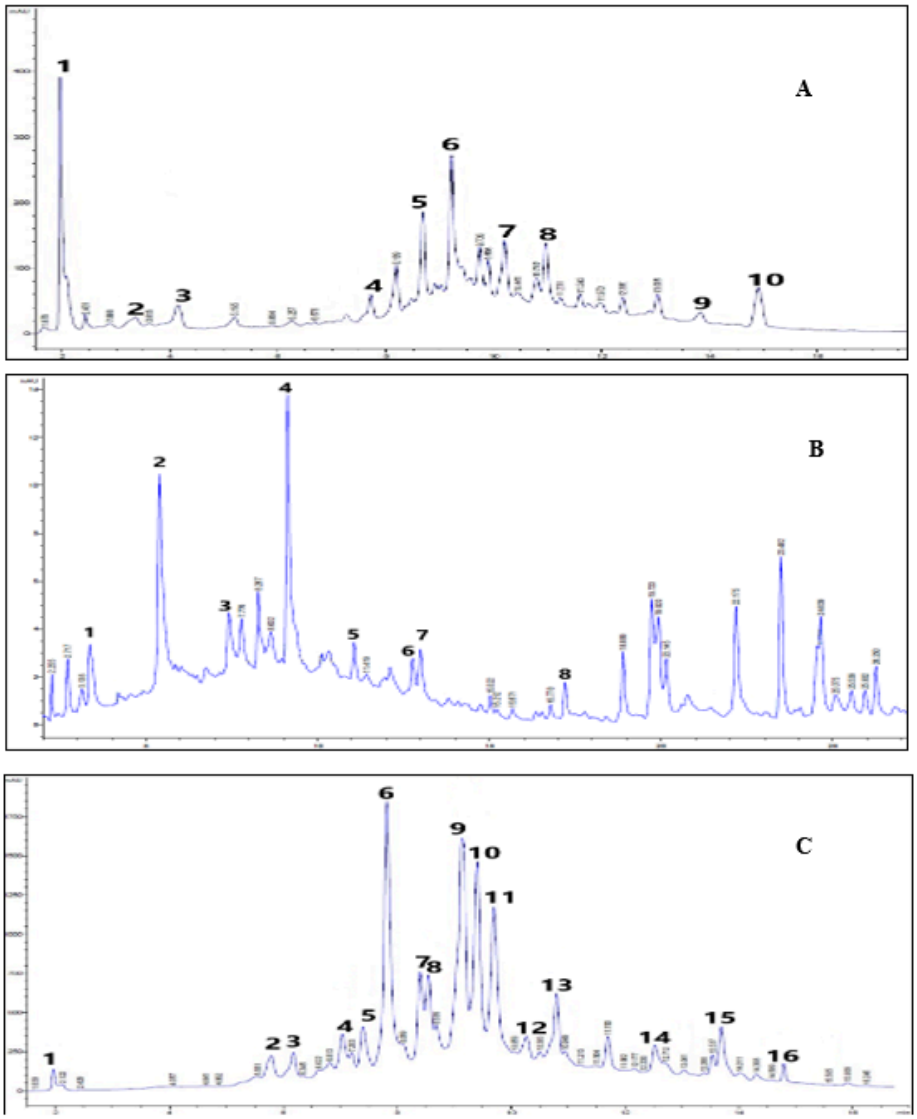


Figure 2

HPLC chromatograms of infusion extracts obtained at 300 nm (**A**): *Ferula vesceritensis*; (**B**): *Salvia verbenaca;* (**C**):*Myrtus communis* L.

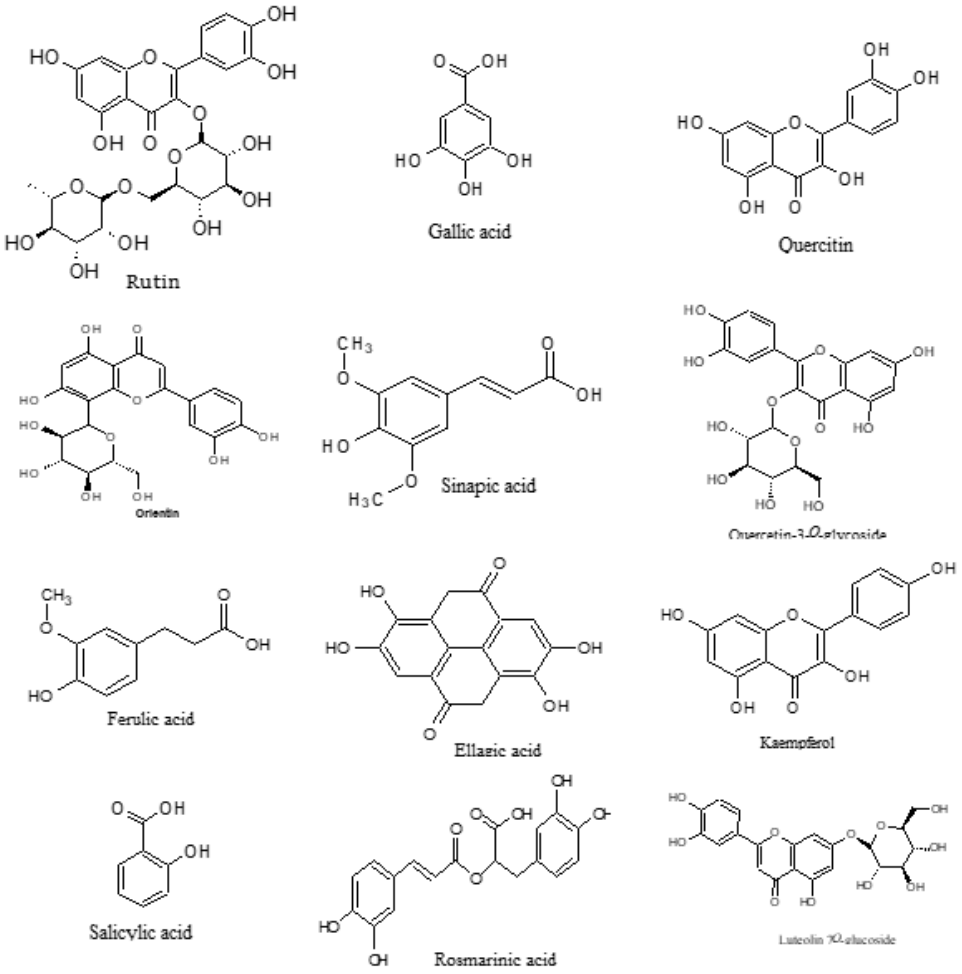


Figure 3

Main phenolic compounds identified in infusion extracts of *Salvia verbenaca*, *Ferula vesceritensis* Coss & Dur. and *Myrtus communis* L.

Supplementary Files

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