

Unveiling the Phytochemical Profiling, hypolipidemic, hypoglycemic and antioxidant effects of different extracts from *Lavandula stoechas* L. (French lavender) grown in Palestine

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ABSTRACT

Background: *Lavandula stoechas* L. (French lavender; Lamiaceae) is a well-known Lavender species in the Mediterranean Sea Basin. It is widely used in traditional medicine owing to its wound healing, antispasmodic, and expectorant properties. As a result, the current study was the first to investigate the phytochemical composition, phenol, tannin, flavonoid contents, DPPH free radicals, porcine pancreatic lipase, and α -amylase inhibitory capacities of *L. stoechas* various polarities fractions from Palestine.

Methods: Specific coloring and precipitation procedures were used for phytochemical screening. Total phenolic content was quantified using the colorimetric technique Folin-Ciocalteu. The aluminum chloride technique was used to determine the total flavonoid level, while the vanillin approach was used to determine tannins. The antioxidant value was determined using the DPPH technique. At the same time, porcine pancreatic lipase and α -amylase inhibitory effects were estimated by p-nitrophenyl butyrate (PNPB) and 3,5-dinitrosalicylic acid (DNSA) approaches, respectively.

Results: The results indicate that *L. stoechas* methanol fraction exhibited the highest total flavonoid content (18.028 ± 1.51 mg of QUE/g) and notable total phenol contents of 127.13 ± 2.07 mg of GAE/g. While acetone fraction yielded the highest total tannin content (94.01 ± 1.08 mg of CAE/g). Moreover, among the *L. stoechas* fractions, the aqueous one has a higher antioxidant ($IC_{50} = 47.86 \pm 0.08$ μ g/ml) compared to methanol ($IC_{50} = 66.06 \pm 0.06$ μ g/ml), acetone ($IC_{50} = 63.09 \pm 0.29$ μ g/ml), and hexane ($IC_{50} = 79.43 \pm 0.1$ μ g/ml) fractions. All plant fractions showed weak porcine pancreatic lipase and α -amylase inhibitory effects compared with the employed positive controls.

Conclusions: The present study has provided valuable insights into the phytochemical composition and bioactivity of different fractions obtained from *L. stoechas*. Based on the results obtained, it is recommended that the specific bioactive compounds responsible for the observed effects be explored and identified.

Keywords: *Lavandulastoechas*; Phytochemical composition; Phenol; Tannin; Flavonoid; DPPH Free Radicals; Porcine pancreatic lipase; α -Amylase.

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INTRODUCTION

Lavandula stoechas L. (French lavender; Lamiaceae) is an evergreen shrub and is considered one of the most commonly known Lavender species, especially in the Mediterranean Sea Basin regions. It is utilized broadly in folk medicine due to its wound healing, carminative, antispasmodic, and expectorant characteristics. *L. stoechas* volatile oil also treats headaches, chest colds, and colics (1). Some investigations have reported the antioxidant (2), antifungal (3), anti-leishmanial (4), anti-inflammatory (5), and antibacterial activities of the plant (6, 7).

The World Health Organization (WHO) reports that the global incidence of obesity has nearly quadrupled since 1975. Moreover, in 2016, about 1.9 billion people aged 18 and older were diagnosed as overweight, with more than 650 million of them classified as obese. These figures demonstrate the enormous and rising global problem of obesity. Obesity has multiple complications that can influence various elements of health. Obesity is associated with a higher risk of cardiovascular illnesses such as stroke and heart disease, respiratory issues such as sleep apnea, musculoskeletal disorders such as osteoarthritis, diabetes, and certain forms of cancer, among other things (8).

According to the WHO, the number of people with diabetes has been rising globally. In 2014, there were 422 million individuals with diabetes, compared to 108 million in 1980. The prevalence of diabetes has been increasing more rapidly in low- and middle-income countries compared to high-income countries. Diabetes is associated with various complications. It is a major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation. These complications significantly impact the health and quality of life of individuals with diabetes (9).

Respiratory illnesses, neurological disorders, cardiovascular diseases, atherosclerosis, metabolic disorders, diabetes, and cancer have all been linked to oxidative stress. It is characterized by excess reactive

oxygen species (ROS), which can cause cellular damage and malfunction (10).

Natural products have been an important source of therapeutic molecules for various medical disorders, including diabetes, obesity, and illnesses caused by oxidative stress (11). Many antidiabetic, anti-obesity, and antioxidative stress pharmaceuticals have their origins in compounds derived from plants, marine organisms, bacteria, fungi, and other natural sources. For example, metformin isolated from *Galega officinalis*, berberine found in *Berberis* species, and Quercetin (found in various fruits and vegetables) (12). Orlistat is a drug used to treat obesity, developed from the lipase inhibitor lipstatin discovered in the *Streptomyces toxytricini* bacteria (13). Furthermore, the catechin Epigallocatechin gallate (EGCG) present in green tea has been researched for its potential to assist in weight control. Moreover, curcumin, generated from turmeric, has potent antioxidative stress (14-16).

Hence, the present research aims to explore the total phenol, tannin, and flavonoid amounts and pancreatic lipase, α -amylase, and free radicals inhibitory effect by *L. stoechas* four extracts.

MATERIAL AND METHODS

Plant material collection, identification, and drying conditions

L. stoechas plant material collection, identification, and drying conditions were followed according to the WHO guidelines on good agricultural and collection practices [GACP] for medicinal herbals (17). However, the plant materials, including the leaves, stems, branches, and flowers of *L. stoechas* shrubs, were collected during the flowering period in May 2023 from the Nablus governorate of Palestine. The taxonomical characteristics were performed in the Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University. The plant was deposited in the Pharmacognosy Laboratory with a voucher number of Pharm-PCT-2802.

The collected plant materials were extensively washed and dried in shady conditions with ordinary humidity for about three weeks. After drying, the dried material was coarsely powdered and kept in glass jars for further experiments.

Instrumentations

Cryo-Desiccator (Mill-rock technology, BT85, Kingston, USA) A Spectrophotometer-UV/Visible (Jenway® 7135, Staffordshire, UK), filter papers (Whitman no.1, Washington, USA), Shaker device (Memmert 531-25-1, Stockholm, Germany), rotavap apparatus (Heidolph-VV 2000, Schwabach, Germany), grinder ([Aero Plus 500W Mixer Grinder](#), I01, Wan Chai, China), and balance-electronic (Radwag, AS 220/c/2, Toruńska, Poland) were used.

Chemicals

Acarbose, p-nitrophenyl butyrate, Orlistat, tris-HCl buffer, and porcine pancreatic lipase type II were sourced from Sigma (St. Louis, USA). Magnesium ribbon, acetic acid, ferric chloride, and DMSO (Dimethyl sulfoxide) were acquired from Riedel De Haen (Teningen, Germany). Folin-Ciocalteu's reagent, hydrochloric acid, aluminum chloride, potassium acetate, chloroform, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Steinheim, Germany).

Furthermore, iodine solution, Sulfuric acid, and Molisch's reagent were sourced from Alfa-Aesar (Lancaster, UK). Ninhydrin solution and Benedict's and Millon's reagents were acquired from Alfa Agar (Binfield, UK). Methanol, n-hexane, acetone, and sodium hydroxide were procured from LobaChemie (Mumbai, India),

Extraction method

The powdered material of the *L. stoechas* plant was sequentially fractionated by adding four solvents of increasing polarity: hexane (non-polar), acetone (polar-aprotic), methanol, and water (polar-protic solvents). About 50 g of the powdered plant material was steeped in 1000 ml of acetone, n-hexane, methanol, and water separately, and each fraction was placed in a shaker for 96 h at room temperature, with 80 rounds per min. Then, each

fraction was kept in a refrigerator for seven days. After that, the hexane, acetone, and methanol fractions were filter-evaporated using a rotavapor under certain vacuum conditions. The aqueous fraction was lyophilized utilizing a Cryo-Desiccator. Finally, all crude plant fractions were stored in the refrigerator at 4 °C until further use (18).

Preliminary phytochemical assessment

The *L. stoechas* plant methanol, water, n-hexane, and acetone fractions were screened for the presence of major natural phytochemical classes by utilizing the phytochemical tests of Trease and Evans (19). They were performed on the organic and aqueous fractions. The alkaloids were highlighted by the reagents of Mayer, Dragendorff, and by the Reagent of Wagner, tannins by ferric chloride, terpenes by the reaction of Liebermann, saponins were determined based on their foam-forming abilities, polyphenolic substances by FeCl_3 , and the revelation of flavonoids by the reaction with cyanidin.

Quantification of phenol

The quantifications of phenolic matter in the *L. stoechas* plant fractions were evaluated using the Folin-Ciocalteu reagent method described previously (20). Using a 100 mL volumetric flask, a 7.5% sodium carbonate (Na_2CO_3) solution was prepared by dissolving 7.5 g of Na_2CO_3 in less than 100 mL of distilled water, and then distilled water was used to bring the volume up to 100 mL. Like sodium carbonate solution, a stock solution of the standard solution (Gallic acid solution) was prepared by dissolving 100 mg of gallic acid and adding up to 100 mL of distilled water. The reaction mixture was prepared by mixing 0.5 mL of each extract solution, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 mL of 7.5% sodium carbonate (Na_2CO_3) in a test tube for each sample. The sample tubes were incubated for 45 min at 45 °C. The absorbance was determined using a spectrophotometer at wavelength 765 nm. The samples were prepared in triplicate for each analytic trial to obtain the mean and standard deviation values.

Quantification of flavonoids

The quantifications of flavonoids in the *L. stoechas* plant

fractions were estimated using the aluminum chloride colorimetric method described previously (21). Quercetin was used to make the standard calibration curve for total flavonoid determination. Stock Quercetin solution was prepared by dissolving 5.0 mg Quercetin in 1.0 mL methanol, then the standard solutions of Quercetin were prepared by serial dilutions using methanol (10–100 mg/mL). An amount of 0.6 mL diluted standard Quercetin solutions or extracts was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against a blank at 420 nm wavelength with UV-Vis spectrophotometer.

Antioxidant assay

The 2,2-di-phenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability was evaluated following the previously described method (22). Each plant extract stock solution was serially diluted to achieve 2-100 µg/mL concentrations using methanol as solvent. Each test tube contained 1 mL of each concentration and was appropriately marked. One mL of 0.002% methanolic DPPH solution was added to each test tube, and 1 mL of methanol was added to each test tube to bring the final volume up to 3 mL (caution: DPPH is light sensitive, so preparation of working test tubes should be performed with minimum light exposure).

The samples were incubated for 30 minutes in a dark place, and then their optical densities were determined using spectrophotometric measurements at a wavelength of 517 nm. The equation used in this analytical study to calculate the inhibition percentage is shown below:

$$\% \text{ DPPH inhibition} = (A_B - A_S) / A_B \times 100\%$$

A_B is the recorded absorbance of the blank solution;
 A_S is the recorded absorbance of the sample solution.

Quantifications of tannin

The condensed tannin contents of four *L. stoechas* fractions were assessed according to Sun *et al.*'s procedure (23). 4% methanolic vanillin solution was prepared freshly. 100 µg/mL stock solution from each plant extract

fraction was prepared using methanol as a solvent. For the working solution, each test tube contained 0.5 mL from each extract mixed with 3 mL of vanillin solution and 1.5 mL of concentrated HCl. The mixture was allowed to stand for 15 min, and then the absorption was measured at 500 nm against methanolic vanillin as a blank. All working samples were analyzed in triplicate. The total tannin in each fraction was expressed as catechin equivalents (mg of CAE/g of plant fraction).

Lipase inhibition assay

The anti-lipase assay was conducted according to Bustanjiet *al.*'s studies (24). A stock solution of 500 µg/mL from each plant fraction, in 10% DMSO, was used to prepare five different solutions with the following concentrations: 50, 100, 200, 300, and 400 µg/mL. A 1 mg/mL stock solution of porcine pancreatic lipase enzyme was freshly prepared in Tris-HCl buffer before be used. The substrate used for this study, *p*-nitrophenyl butyrate (PNPB), was prepared by dissolving 20.9 mg in 2 mL of acetonitrile. For each working test tube, 0.1 mL of porcine pancreatic lipase (1 mg/mL) was mixed with 0.2 mL of each diluted solution series for each plant fraction. The resulting mixture was then brought to a total volume of 1 mL, by adding Tri-HCl solution and incubated at 37 °C for 15 min. Following incubation, 0.1 mL of PNPB solution was added to each test tube. The mixture was incubated for 30 min at 37 °C. Pancreatic lipase activity was determined by measuring the hydrolysis of the PNPB compound into *p*-nitrophenolate ions at 410 nm using a UV spectrophotometer. The same procedure was repeated for Orlistat, which was used as a standard reference compound. The equation used in this analytical study is shown below:

$$\% \text{ Lipase inhibition} = (A_B - A_S) / A_B \times 100\%$$

A_B is the recorded absorbance of the blank solution;
 A_S is the recorded absorbance of the sample solution.

α -Amylase inhibitory assay

This method was carried out by utilizing the procedure of McCue and Shetty (25). For working solutions, a volume of 0.2 mL of enzyme solution was mixed with 0.2 mL of each plant extract fraction and was incubated for

10 min at 30 °C. After the incubation period, 0.2 mL of a freshly prepared 1% starch aqueous solution was added to each working solution, followed by an incubation period of at least 3 min. The reaction was stopped by the addition of 0.2 mL dinitrosalicylic acid (DNSA) yellow color reagent. Each working solution was then diluted with 5 mL of distilled water and then boiled for 10 min in a water bath at 90 °C. The mixture was cooled to room temperature, and the absorbance was taken at 540 nm. The blank was prepared following the steps above, but the plant fraction was replaced with 0.2 mL of the previously described buffer. Acarbose was used as the standard reference, following the same steps used for plant extract.

Statistical analysis

All of the obtained results of the four studied plant fractions (quantifications of tannin, phenols, and flavonoids, in addition to the antioxidant, anti-lipase, and anti-amylase

activities) were repeated three times for each experiment and expressed as means $\pm$ SD standard deviation. When the *p-value* was <0.05 , the outcomes were considered significant. Data were compared using unpaired *t*-tests.

RESULTS

Phytochemical screening

The results of the phytochemical preliminary tests on the *L. stoechas* aqueous fractions revealed the presence of cardiac glycosides, phenols, and flavonoids. Meanwhile, phenols, tannins, and flavonoids were observed in the methanol fraction. At the same time, tannin, phenols, flavonoids, steroids, and volatile oils were identified in the acetone fractions. Moreover, phenols, flavonoids, steroids, carbohydrates, and volatile oils were found in the plant hexane fraction, as depicted in Table 1, and the fractionation yields are shown in Table 2.

Table 1 Phytochemical screening assessment of *L. stoechas* four solvents fractions

Phytochemical Classes	Aqueous fraction	Methanol fraction	Acetone fraction	Hexane fraction
Cardiac glycosides	+ve	-ve	-ve	-ve
Saponin glycoside	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve
Protein	-ve	-ve	-ve	-ve
Starch	-ve	-ve	-ve	-ve
Phenols	+ve	+ve	+ve	+ve
Volatile oil	-ve	-ve	+ve	+ve
Tannins	-ve	+ve	+ve	-ve
Steroids	-ve	-ve	+ve	+ve
Reducing sugar	-ve	-ve	-ve	-ve
Carbohydrate	-ve	-ve	-ve	+ve
Flavonoids	+ve	+ve	+ve	+ve

Positive (+ve); Negative (-ve)

Table 2 The yield percentage of *L. stoechas* fractions

Fractions	Plant extract, (g)	Dried plant, (g)	Yields, (%)
Hexane	4.55	50	9.10
Acetone	2.71	50	5.42
Methanol	3.82	50	7.64
Aqueous	3.11	50	6.22

Quantitative analysis of phenols

To determine the total phenol content in the *L. stoechas*

plant, Table 3 displays the absorbance values for various concentrations of the Gallic acid standard.

Table 3. Absorption values of several concentrations of the standard Gallic acid

Gallic acid concentrations (mg/ml)	Absorption at $\lambda_{\max}$ =765 nm
0	0.00
10	0.14
40	0.50
50	0.56
70	0.80

From the calibration curve of Gallic acid, the following equation was calculated to estimate the total phenol content in the four *L. stoechas* plant fractions.

$$y = 0.0112x + 0.0176, R^2 = 0.9956$$

Where y is the absorbance at 765nm, and x is the total

phenol content of the *L. stoechas* plant fraction.

Absorbance values from various concentrations were employed to determine the total flavonoid contents in four fractions of *L. stoechas*. A standard calibration curve was constructed to quantify the total flavonoid content in these fractions. Table 4 presents the absorbance values for different concentrations of the standard Quercetin.

Table 4. Absorption values of several concentrations of the standard Quercetin

Concentration of quercetin (mg/mL)	Absorption at $\lambda_{\max}$ =510 nm
0	0
10	0.01
30	0.01
50	0.02
70	0.03
100	0.04

Using the standard calibration curve of Quercetin, the equation $y = 0.0004x + 0.0011$ with an R^2 value of 0.995 was applied to determine the total flavonoid contents in the four fractions of the *L. stoechas* plant.

Where Y is the absorbance at 510 nm, and X is the total

flavonoids in the plant fractions. Furthermore, to assess the total tannin contents in the four fractions of the *L. stoechas* plant, the absorbance values of various concentrations of the standard Catechin were examined and are detailed in Table 5.

Table 5. Absorption values of several concentrations of the standard Catechin

Concentration of Catechin (μ g/mL)	Absorption at 500 nm $\lambda_{\max}$
0	0
10	0.08
30	0.11
50	0.15
70	0.18
100	0.29

Utilizing the standard calibration curve of Catechin, the equation $y = 0.0025x + 0.0232$ with an R^2 value of 0.9569 was employed to estimate the total condensed tannin contents in the four fractions of the *L. stoechas*

plant. Here, Y represents the absorbance at 500 nm, and X denotes the total tannin contents in the four fractions of the *L. stoechas* plant.

The investigation into the phytochemical composition

of different *L. stoechas* plant fractions, extracted using varying solvents, revealed distinct profiles regarding total flavonoids, phenols, and tannins. The data, presented as

mean values with standard deviations ($\pm$ SD), are summarized in Table 6.

Table 6. Total flavonoids, tannins, and phenols of *L. stoechas* the aqueous, methanol, hexane, and acetone fractions.

Fractions	Total flavonoids contents, mg of QUE/g of plant fraction, $\pm$ SD	Total phenol contents, mg of GAE/g of plant fraction, $\pm$ SD	Total Tannin contents, mg of CAE/g of plant fraction, $\pm$ SD
Hexane	10.01 $\pm$ 1.22	36.04 $\pm$ 1.01	-
Acetone	11.011 $\pm$ 1.18	73.07 $\pm$ 2.02	94.01 $\pm$ 1.08
Methanol	18.028 $\pm$ 1.51	127.13 $\pm$ 2.07	37.04 $\pm$ 1.11
Aqueous	16.02 $\pm$ 0.61	88.09 $\pm$ 2.04	-

The experiment was repeated in triplicate. P-value less than 0.05

The results indicate that the methanol fraction exhibited the highest total flavonoid content (18.028 $\pm$ 1.51 mg of QUE/g), suggesting its effectiveness in extracting flavonoids from *L. stoechas*. The aqueous fractions showed a relatively lower value of 16.02 mg of QUE/g.

In addition, *L. stoechas* methanol fraction demonstrated notable total phenol contents of 127.13 $\pm$ 2.07 mg of GAE/g, followed by aqueous and acetone plant fractions (88.09 $\pm$ 2.04 and 73.07 $\pm$ 2.02 mg of GAE/g, respectively). Hexane exhibited the lowest phenolic content, with 36.04 $\pm$ 1.01 mg of GAE/g values.

Moreover, *L. stoechas* acetone extraction yielded the highest total tannin content (94.01 $\pm$ 1.08 mg of CAE/g), followed by methanol (37.04 $\pm$ 1.11 mg of CAE/g). Meanwhile, hexane and aqueous fractions did not show detectable tannin content.

The variations observed in the phytochemical composition across the fractions highlight the solvent-dependent extraction efficiency. Water and methanol appear to be effective solvents for extracting phenolic and acetone, and methanol seems to be effective solvents for extracting tannin compounds. In contrast, methanol is particularly efficient in extracting flavonoids. These findings contribute valuable insights for the strategic selection of solvents in future studies focusing on the medicinal properties of *L. stoechas*.

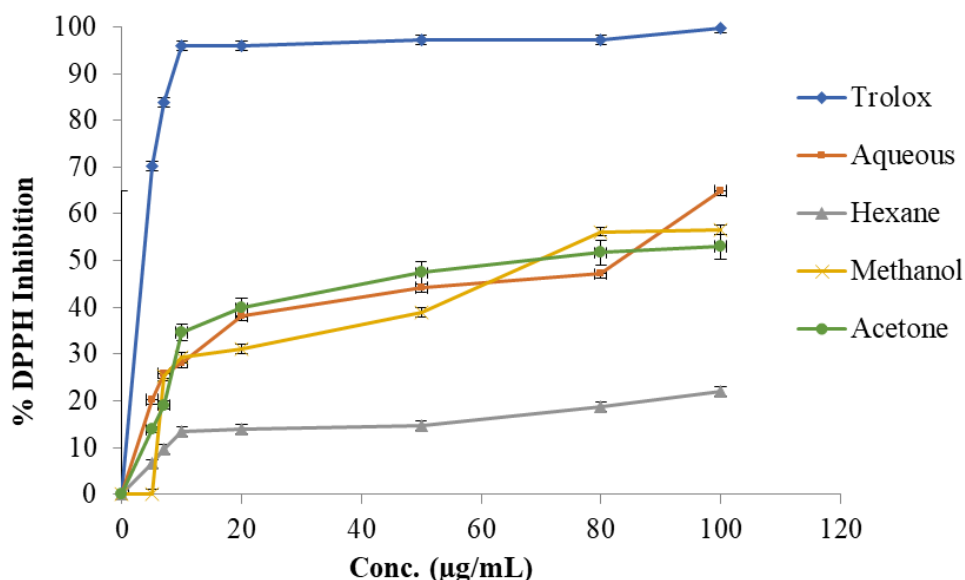
Antioxidant effect

Thus, the DPPH technique was utilized in this study to examine the in vitro antioxidant activity of the *L. stoechas* hexane, acetone, methanol, and aqueous fractions. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used in antioxidant research to evaluate compounds' free radical scavenging activity. The advantages of the DPPH technique include being widely adopted and standardized, broad applicability, and low cost (26). In addition, the DPPH assay provides quantitative results, allowing researchers to compare the antioxidant activity of different compounds or samples. Moreover, this assay is sensitive to both hydrophilic and lipophilic antioxidants, allowing for the assessment of the total antioxidant capacity of a sample. Also, DPPH is a stable free radical, which contributes to the reproducibility of the assay and can be applied to a wide range of samples, including natural extracts, synthetic compounds, and even food samples. Most importantly, the DPPH assay is a simple and rapid method that can be easily conducted in a laboratory setting (27, 28).

The DPPH percentage of inhibition and IC₅₀ doses were determined. The antioxidant IC₅₀ values, % DPPH inhibition of *L. stoechas* four fractions, and positive control (Trolox) are presented in Fig. 4 and Table 7.

Table 7. DPPH inhibition percentages and IC₅₀ values (µg/mL) of *L. stoechas* different fractions and Trolox

Conc.	Trolox, ±SD	Aqueous fraction, ±SD	Methanol fraction, ±SD	Acetone fraction, ±SD	Hexane fraction, ±SD
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
5	70.2±0.29	20.25±0.05	25.3±0.2	13.855±0.05	6.49±0.05
7	83.95±0.58	25.85±0.1	29.35±0.05	19.08±0.03	9.55±0.1
10	95.99±1.22	28.17±0.05	31.13±0.04	34.6±0.01	13.4±0.15
20	96±1.62	38.1±0.12	38.85±0.05	39.85±0.025	13.89±0.05
50	97.15±2.1	44.2±0.15	56.2±0.09	47.39±0.21	14.75±0.15
80	97.33±1.52	47.19±0.05	56.65±0.09	51.7±0.7	18.65±0.12
100	99.71±2.2	64.94±0.05	57.59±0.05	53.02±0.22	22.09±0.05
IC ₅₀ , ±SD	2.81±1.54	47.86±0.08	66.06±0.06	63.09±0.29	79.43±0.1

**Figure 4. DPPH radical scavenging activity of *L. stoechas* plant fractions and Trolox. The experiment was repeated in triplicate. P-value less than 0.05**

Trolox, a standard antioxidant, demonstrated a concentration-dependent increase in DPPH radical scavenging activity. At the highest concentration (100 µg/ml), Trolox exhibited a remarkable inhibition percentage of 99.71±2.2%, indicating potent antioxidant capacity.

Among the fractions of *L. stoechas*, the aqueous fraction showed a progressive increase in antioxidant

activity with concentration. The methanol and acetone fractions also exhibited concentration-dependent activities, reaching inhibition percentages of 57.59±0.05% and 66.06±0.06%, respectively, at the highest concentration. While demonstrating lower activity at lower concentrations, the hexane fraction substantially increased at higher concentrations.

The IC₅₀ values represent the concentration at which

50% inhibition of DPPH radicals occurs. Trolox had the lowest IC₅₀ (2.81±1.54 µg/ml), suggesting its superior efficacy. Among the fractions, the aqueous fraction had a higher IC₅₀ (47.86±0.08 µg/mL) compared to methanol (66.06±0.06 µg/ml), acetone (63.09±0.29 µg/ml), and hexane (79.43±0.1 µg/ml) fractions.

α-Amylase inhibition assay

An *in vitro* assay of α-amylase inhibitory activity using starch as a substrate and Acarbose as a positive control was

conducted on four *L. stoechas* plant fractions. The α-amylase inhibitory activity of Acarbose and *L. stoechas* fractions was assessed at various concentrations (0, 10, 50, 70, 100, and 500 µg/ml) using different solvents (Aqueous, Methanol, Acetone, and Hexane). The results of IC₅₀ α-amylase inhibitory activity values for the four fractions of *L. stoechas* and Acarbose are illustrated in Table 8 and were obtained from Figure 5.

Table 8. α-Amylase inhibitory activity of Acarbose and *L. stoechas* fractions

Conc.	Acarbose±SD	Aqueous±SD	Methanol±SD	Acetone±SD	Hexane±SD
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
10	53.22±0.29	6.6±0.1	2.36±0.02	8.85±0.02	8.86±0.02
50	54.91±0.58	8.66±0.14	8.85±0.02	9.5±0.03	9.51±0.02
70	66.1±1.22	9.715±0.025	31.4±0.2	17.83±0.13	15.87±0.08
100	66.1±1.62	33.47±0.07	33.76±0.00	24.1±0.1	27.94±0.03
500	72.54±2.1	34.61±0.02	36.95±0.06	32.85±0.02	34.08±0.15
IC ₅₀ (µg/mL), ±SD	28.18±0.38	12.5 × 10 ³ ±0.07	11.7 × 10 ³ ±0.07	19.5 × 10 ³ ±0.06	19.9 × 10 ³ ±0.07

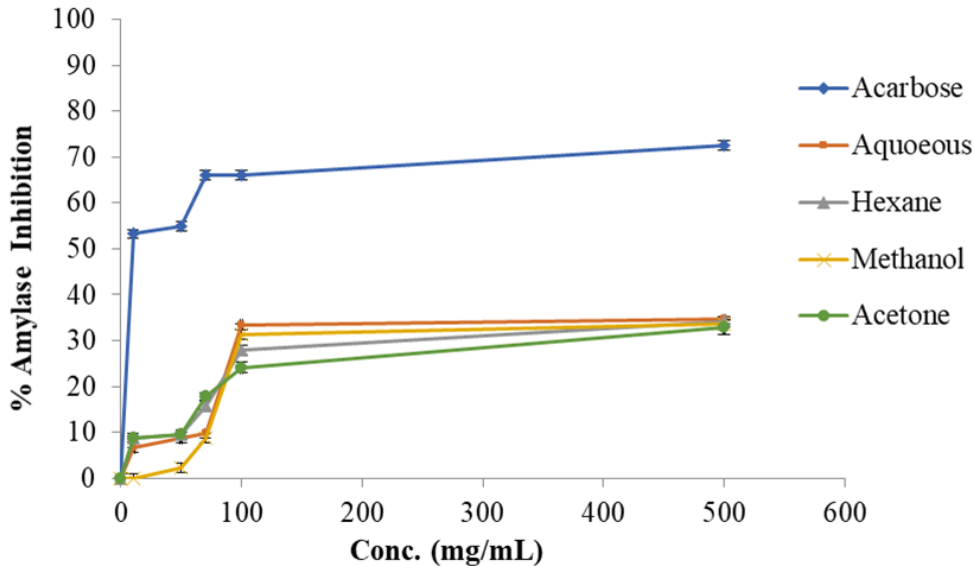


Fig. 5 α-Amylase inhibitory activity values of Acarbose and *L. stoechas* fractions. The experiment was repeated in triplicate. P-value less than 0.05

Both Acarbose and *L. stoechas* fractions exhibited concentration-dependent α-amylase inhibitory activity. As

the concentration increased, the inhibitory activity also increased, reaching its maximum at the highest

concentration (500 $\mu\text{g/mL}$). Acarbose demonstrated substantial inhibitory activity at all concentrations, with a notable increase at higher concentrations. *L. stoechas* fractions also showed inhibitory effects, albeit generally lower than Acarbose.

The choice of solvent significantly affected the α -amylase inhibitory activity. Among the solvents used, methanol and aqueous extracts of *L. stoechas* displayed higher inhibitory potential ($\text{IC}_{50}=11.7 \times 10^3 \pm 0.07$ and $12.5 \times 10^3 \pm 0.07$, respectively) compared to acetone and hexane extracts ($\text{IC}_{50}=19.5 \times 10^3 \pm 0.06$ and $19.9 \times 10^3 \pm 0.07$, respectively).

Porcine pancreatic lipase enzyme inhibition activity

The hydrolysis of *p*-nitrophenyl butyrate to *p*-nitrophenol was used to measure the influence of the four *L. stoechas* fractions on the porcine pancreatic lipase enzyme. The assay worked by comparing a strong lipase inhibitory agent to Orlistat. The lipase enzyme inhibitory activity of four *L. stoechas* fractions and Orlistat. The lipase inhibitory results of four *L. stoechas* fractions and Orlistat in their IC_{50} values are shown in Table 9 and Figure 6.

Table 9. Lipase inhibition activity and IC_{50} ($\mu\text{g/mL}$) doses of *L. stoechas* four fractions and Orlistat

Conc.	Orlistat	Aqueous	Methanol	Acetone	Hexane
0	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00
10	62.5 $\pm$ 0.8	7.3 $\pm$ 0.01	4.33 $\pm$ 0.54	4.86 $\pm$ 0.01	2.14 $\pm$ 0.03
50	66 $\pm$ 0.58	11.66 $\pm$ 0.01	15.89 $\pm$ 0.09	11.66 $\pm$ 0.015	7.56 $\pm$ 0.02
100	94.45 $\pm$ 1.5	23.04 $\pm$ 0.02	43.8 $\pm$ 0.1	15.845 $\pm$ 0.14	10.45 $\pm$ 0.03
500	97.3 $\pm$ 1.22	43.8 $\pm$ 0.1	43.81 $\pm$ 0.1	19.23 $\pm$ 0.01	15.66 $\pm$ 0.06
700	98.25 $\pm$ 0.58	45.75 $\pm$ 0.03	44.51 $\pm$ 0.00	26.83 $\pm$ 0.27	17.77 $\pm$ 0.13
IC_{50} ($\mu\text{g/mL}$), $\pm$ SD	12.3 $\pm$ 0.97	$1.5 \times 10^3 \pm 0.05$	$3.16 \times 10^3 \pm 0.07$	$79 \times 10^3 \pm 0.14$	$15.8 \times 10^3 \pm 0.06$

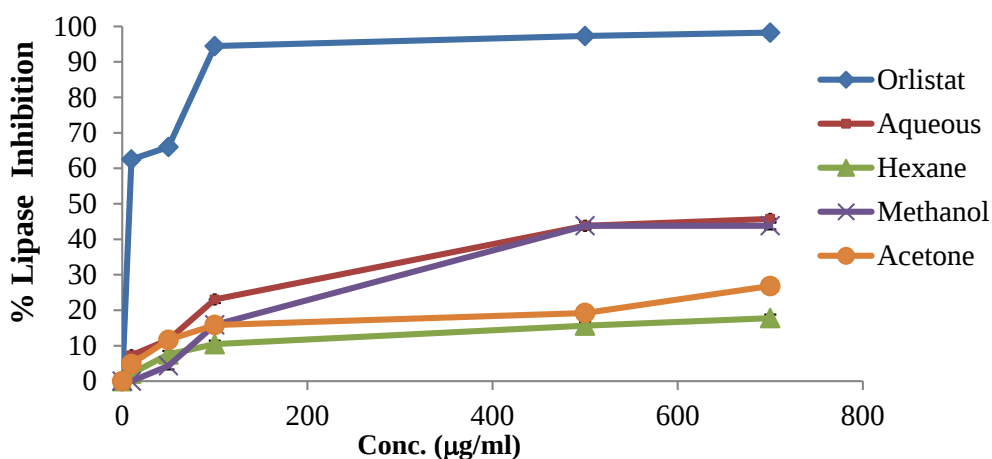


Fig. 6. Porcine pancreatic lipase inhibition activity of Orlistat and *L. stoechas* fractions. The experiment was repeated in triplicate. P-value less than 0.05

The lipase inhibition activity of the fractions obtained from *L. stoechas* was compared with the standard lipase

inhibitor, Orlistat. The results indicate that all fractions of *L. stoechas* exhibited dose-dependent lipase inhibition. As

the concentration increased, a corresponding increase in inhibitory activity was observed for each fraction.

Among the fractions, the aqueous fraction demonstrated the highest lipase inhibition activity, especially at higher concentrations. The IC₅₀ values further highlight the potency of the aqueous fraction, with a notably lower IC₅₀ than the other fractions. This suggests that the aqueous fraction of *L. stoechas* may contain bioactive compounds with strong lipase inhibitory properties.

DISCUSSION

The phytochemical screening for *L. stoechas* in our study agrees with the study of Baptista et al. concerning polyphenols and flavonoids (25), with Ezzoubi et al. for tannins, catechin tannins, Flavonoids, and sterols (26), Teixeira et al. for polyphenols, flavonoids and terpenes (27). Moreover, the phytochemical screening of *L. stoechas* reveals that these plant extracts are rich in bioactive compounds that contribute to their medicinal properties (28-29). Advanced analytical techniques further enhance our understanding of *L. stoechas* chemical composition, paving the way for harnessing its therapeutic benefits in various health and wellness products.

Ceylan et al. stated that *L. stoechas* methanol extract from Turkey had DPPH free radical scavenging inhibitory activity by $84.45 \pm 5.1\%$ (29).

In addition, Zoubi et al. investigated the DPPH scavenging activity of *L. stoechas* from Morocco and found that its hexane extract had potent antioxidant activity with an IC₅₀ value of 1.2 µg/mL (30).

In 2012, Robu et al. performed a comparative study of antioxidant activity for different *L. angustifolia* and *L. hybrida* cultivars. The highest result was obtained for *L. hybrida* followed by *L. angustifolia* (30). On the other hand, Blazeković et al. found a slightly higher antioxidant activity was observed for *L. angustifolia* extracts than *L. intermedia* extracts, possibly due to their higher polyphenolic contents (31). Ahn et al. found that the

aqueous-methanolic leaf extract of *L. angustifolia* afforded over twice as high DPPH value as the *L. latifolia* extract (32). The antioxidant, antidiabetic, and antilipase effects of *L. stoechas* are consistent with other studies on medicinal plants in Palestine. For example, Rahhal et al. 2022 found that the *A. scoparia* aqueous extracts had promising antioxidant, anti-lipase, and anti-α-amylase effects (33).

CONCLUSION

This study is the first one in Palestine, and the results indicate that the *L. stoechas* methanol fraction exhibited the highest total flavonoid and phenol contents. At the same time, the acetone fraction yielded the highest total tannin content. Moreover, among the *L. stoechas* fractions, the aqueous one has a higher antioxidant capacity. All plant fractions showed weak anti-lipase and α-amylase inhibitory effects compared with the employed positive controls. This study has provided valuable insights into the phytochemical composition and bioactivity of different fractions obtained from *L. stoechas*. Based on the results obtained, it is recommended that the specific bioactive compounds responsible for the observed effects need to be isolated, purified, and identified. Further studies involving *in vivo* models and clinical trials are warranted to validate the potential health benefits of *L. stoechas* fractions. Legal procedures by the stakeholders in the country are required to start research for the application of medicinal plants in complementary medicine.

Statements and Declaration

Availability of data and materials

This published article and its supplementary information files include all data generated or analyzed during this study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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None

Authors' contributions

B. R.: Conceptualization, Validation, Investigation, writing – original draft, Writing - review & editing, Visualization, Supervision, Project administration. **N. J.:** Conceptualization, Validation, Investigation, writing – original draft, Writing - review & editing, Visualization, Supervision, Project administration. **L. I.:** Validation, Investigation, Writing - review & editing, Visualization,

Supervision. **F. H.:** Validation, Investigation, Writing - review & editing, Visualization. **L. G.:** Validation, Investigation, Writing - review & editing, Visualization. **S. A.:** Validation, Investigation, Writing - review & editing. **W. J.:** Validation, Investigation, Writing - review & editing. **Z. B.:** Validation, Investigation, Writing - review & editing. **Z. H.:** Validation, Investigation, Writing - review & editing. **Z. H.:** Validation, Writing - review & editing. All authors read and approved the final manuscript.

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دراسة المحتوى الكيميائي، وتأثيرات خفض مستوى الدهون و مستوى السكر في الدم، ومضادات الأكسدة لمستخلصات مختلفة من نبات اللافندر الفرنسي (*Lavandula stoechas* L) المزروع في فلسطين

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ملخص

Lavandula stoechas L. (الخزامى الفرنسي؛ Lamiaceae) نوعٌ معروف من الخزامى في حوض البحر الأبيض المتوسط. يُستخدم على نطاق واسع في الطب التقليدي نظرًا لخصائصه في التئام الجروح، وخصائصه المضادة للتشنج، وخصائصه المقشعة. ونتيجةً لذلك، تُعد هذه الدراسة الأولى التي تبحث في التركيب الكيميائي النباتي، ومحتويات الفينول، والتانين، والفلافونويد، والجذور الحرة DPPH، والليبيز البنكرياسي الخنزيري، والقدرات المثبطة لـ α -amylase لأجزاء مختلفة من *L. stoechas* من فلسطين.

الطريقة: استُخدمت أساليب تلوين وترسيب محددة للفحص الكيميائي النباتي. حُدد محتوى الفينول الكلي باستخدام تقنية قياس الألوان Folin-Ciocalteu. واستُخدمت تقنية كلوريد الألومنيوم لتحديد مستوى الفلافونويد الكلي، بينما استُخدمت طريقة الفانيلين لتحديد العفص. وحُددت القيمة المضادة للأكسدة باستخدام تقنية DPPH. وفي الوقت نفسه، قُدرت التأثيرات المثبطة لليبيز البنكرياسي الخنزيري وألفا أميليز باستخدام طريقتي بارا-نيتروفينيل بوتيرات (PNPB) وحمض 5-داينيتروساليسيليك (DNSA)، على التوالي.

النتائج: تشير النتائج إلى أن نسبة الميثانول في *L. stoechas* أظهرت أعلى محتوى إجمالي من الفلافونويد (18.028 $\pm$ 1.51 ملغ من QUE/غ) ومحتوى إجمالي ملحوظ من الفينول بلغ 127.13 ± 2.07 ملغ من GAE/غ، بينما أظهرت نسبة الأسيتون أعلى محتوى إجمالي من التانين (1.08 $\pm$ 94.01 ملغ من CAE/غ). علاوة على ذلك، من بين نسب *L. stoechas*، تتميز النسبة المئوية بـ IC50 أعلى (0.08 $\pm$ 47.86 ميكروغرام/مل) مقارنةً بنسب الميثانول (66.06 $\pm$ 0.06 ميكروغرام/مل) والأسيتون (0.29 $\pm$ 63.09 ميكروغرام/مل) والهكسان (0.1 $\pm$ 79.43 ميكروغرام/مل). أظهرت جميع أجزاء النبات تأثيرات ضعيفة في تثبيط الليبيز البنكرياسي الخنزيري وألفا أميليز مقارنةً بالضوابط الإيجابية المستخدمة.

الخلاصة: قدمت هذه الدراسة رؤى قيمة حول التركيب الكيميائي النباتي والنشاط الحيوي لأجزاء مختلفة من *L. stoechas*. بناءً على النتائج المُحصل عليها، يُوصى باستكشاف وتحديد المركبات النشطة بيولوجيًا المسؤولة عن التأثيرات الملحوظة.

الكلمات الدالة: لافاندولاستويكاس؛ التركيب الكيميائي النباتي؛ الفينول؛ التانين؛ الفلافونويد؛ الجذور الحرة DPPH؛ الليبيز البنكرياسي الخنزيري؛ ألفا أميليز.

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