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SHORT COMMUNICATION

Phenolic Content, Antioxidant, Hemidiaphragm Glucose Consumption, and Hemoglobin Glycosylation Inhibitory Activities of *Lavandula stoechas* L. Aqueous Extract

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Abstract

The aqueous extract of *Lavandula stoechas* (AqLs) is employed as a diabetic cure in Eastern Moroccan traditional medicine. The aim of this study was to confirm and search for the antidiabetic mechanisms of this plant. The goal of this research is to look into the *in vitro* antioxidant activity of *L. stoechas's* aqueous extract which was analyzed by using two different techniques; 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and β-carotene bleaching assay, with an IC50 = 0.031 ± 0.02 mg/mL and an IC50 = 94.33 ± 12.5 µg/ml respectively. Furthermore, the polyphenolic and flavonoid concentrations were calculated at 146.71 ± 0.53 mg GAE/mg of AqLs, and 721,21 ± 0,21 µg QE/mg of AqLs respectively. Besides, the *in vitro* research of glucose consumption by Peripheral glucose consumption reveals that the combination of this extract, plus insulin, enhances the activity of insulin and improves glucose utilization by the hemidiaphragm with 166.89 ± 23.56 mg/g/h. Finally, the *in vitro* hemoglobin glycosylation test validated *L. stoechas* antidiabetic efficacy with activity equal to 48.94 ± 3.67 mg/mL compared to the gallic acid. Consequently, the aqueous extract of *L. stoechas* was discovered to have promising antidiabetic and antioxidant properties in this research, which can be considered for more biological exploration.

Keywords: Antidiabetic, Antioxidant, Hemidiaphragm, Glycosylation, *Lavandula stoechas*

1. Introduction

Diabetes Mellitus (DM) is a very prevalent abnormality in carbohydrates, proteins, and fats metabolism represented by hyperglycemia[1]. Consequently, this illness was divided into two categories, referred to as diabetes Type I, which is characterized by a deficit in insulin production, because of the damage of the pancreatic β-cells. And, diabetes Type II, which is characterized by insulin-activity resistance, this classification has been acknowledged throughout Ibn Sinaa's book "The Canon of Medicine", in which he discussed it[1]. This high glucose level has the ability to cause either microvascular like renal failure and neurological issues or macrovascular like cerebrovascular and coronary artery diseases[2]. Therefore, diet, exercise, synthetic oral hypoglycemic medicines, and/or insulin can all help to control this illness[3]. And, despite the appearance of new therapeutic molecules such as insulin or oral hypoglycemic agents such as biguanides and sulfonylurea, their regular administration generates several adverse effects. Prolonged insulin exposure lowers the number of receptors in cell surfaces, which also can lead to many complications[4]. Traditional remedies may consist of a variety of active ingredients or molecules that can alter different biological processes and treat diabetes symptoms through a variety of mechanisms, offering diverse advantages[5]. Hence, traditional medicine has served as a source of medical items, and there have been

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numerous initiatives throughout the years to employ herbal medications to treat diabetes[6]. This employment may be due to several modes of action; such as the impact on glucose absorption and glucose transporters, the inhibition of enzymes' (α-glucosidase and α-amylase) activity, the improvement of insulin production, the inhibition of PTPN1's action, the enhancement of pancreatic β-cell proliferation, and the antioxidant capacity[7]. Among these plants, there is *Lavandula stoechas* L., a well-known ethnomedicinal plant, which belongs to the Lamiaceae family[8]. It has several names according to the region where it exists. In Arabic and Berber, we call it *halhal* and *tigaršt*[9]. It can be found in abundance in Africa, Asia, and Europe. It has spread throughout the Middle East, especially in the northeast of Morocco[10]. In traditional medicine, according to the research, it is used to treat several diseases including headaches, eczema, ear infections, cholesterol, and digestive system illnesses, and especially to treat diabetes (Type I and II)[9]. In Morocco, it's usually employed as a decoction or infusion of the aerial part for its antidiabetic effect[11]. The essential oil content of *L. stoechas* has been specifically linked to a number of activities in earlier investigations. As a result, numerous research has looked closely at the biological actions of essential oils and their composition. Aqueous extracts made from the plant's aerial portions, though, have also been reported to exhibit considerable biological activity, according to research[12]. For example, the plant's ethanolic and aqueous extracts both exhibited strongly *in vitro* antioxidant properties[13]. Additionally, when the essential oil was removed from the aerial parts, a decoction was employed in traditional medicine to lower blood cholesterol levels[14].

To that end, *in vitro* antioxidant, the bioavailability of important phenolic compounds *in vitro* and anti-diabetic properties of the aqueous extract of *L. stoechas* were examined in this study by different tests including Hemoglobin glycosylation test and peripheral glucose consumption test.

2. Materials and Methods

2.1 Reagents and Chemicals

The aluminum chloride ($AlCl_3$), DPPH, Folin-Ciocalteu, quercetin, methanol, ascorbic acid, β-carotene, gallic acid and Tween-80, were obtained from Sigma-Aldrich (Steinheim, Germany). Glucose was purchased from Biosystems, Spain. The sodium bicarbonate ($NaHCO_3$) was purchased from Sigma-Aldrich (France).

2.2 Plant Material

Lavandula stoechas was first obtained from *Tafoughalt* (Oriental, Morocco) in the autumn of November, 2019. The plant sample was deposited in the Herbarium of Mohamed First University's Faculty of Sciences in Oujda, Morocco, and identified by a botanist using the reference number (HUMPOM77). Whereby, it has been through two key procedures in order to reach its final state, which is *L. stoechas* powder; the first action was to use distilled water to properly clean the plant's aerial area of any dirt or unwanted particles, and then dry it completely at 40°C in an oven before cutting the plant into little parts. After boiling 80 g of the used plant for 20 mins in distilled water (800 mL), the aqueous extract of the plant was made with the decoction method for the next procedure. Finally, the decoction mixture was filtered and dehydrated at 40°C to produce the desired form, which is the powder of the plant.

2.3 Determination of Total Phenolic Concentration

The total phenolic contents in *L. stoechas* aqueous extract was evaluated using the Folin-Ciocalteu technique[15]. A mixture of Folin-Ciocalteu reagent (1000 µl) and an aqueous extract of *Lavandula stoechas* (200 µl) was used. After a 5 mins ambient-temperature incubation time, 800 µl of sodium carbonate mixture (75 g/l) was added into the solution. After properly stirring all samples, and incubating for 60 mins, absorbance was determined at a wavelength of 765 nm. A calibration curve was produced using gallic acid. A blank consisting of methanol (200 µl), Folin-Ciocalteu reagent (1000 µL), and sodium carbonate aqueous solution (800 µl) (75 g/l). The total polyphenol concentration was calculated in µg gallic acid/mg plant extract.

2.4 Flavonoid Content Determination

The formation of a flavonoid-aluminum complex was the technique used to determine the flavonoid content of *L. stoechas* extract, according to Chen *et al.*,[16], with some modifications. In tubes, we put 200 µl of *L. stoechas* extract at a concentration of 0.5 mg/ml, then we add 1000 µl of distilled water with sodium nitrate (50 µl) (5% w / v). Six minutes later, aluminum chloride (120 µl) (10% w/v) was

included. Following a five-minutes incubation period, 400 µl of NaOH (1M) was included, reaching 2000 µl in the total volume. Finally, at 430 nm, the absorbances were measured, versus a blank made up of 200 µl of distilled water, 120 µl of aluminum chloride ($AlCl_3$, 10%), and sodium nitrate (50 µl). A calibration curve was produced using quercetin. The total flavonoid concentration was calculated in µg quercetin/mg plant extract.

2.5 Free Radical Scavenging Action, DPPH

DPPH is a well-known radical that is used to estimate the antioxidant activity of extract components by evaluating their capacity to provide electrons and hydrogen atoms[17]. *AqLs*'s Radical scavenging action was evaluated using the method described by Chu *et al.*,[18] with some changes. The antioxidant activity of the AqLs was examined at increasing doses varying between 0.312 to 4 mg/ml. For every case 0.2 mL of the test extract was combined with 1.5 mL of 2% DPPH methanolic solution (2 mg/100). After homogenization, the incubation of samples was done in the dark at room temperature for half an hour before being analyzed by a spectrophotometer at a wavelength of 517 nm. Ascorbic acid is used as a reference. All of the experiments were carried out in triple.

The per cent of free radical inhibitory efficacy was determined using the formula:

$$\% \text{ Of inhibition} = (A_{DPPH} - \frac{A - A_{Test}}{A_{DPPH}}) \times 100$$

where,

A_{DPPH} : DPPH solution absorbance in the absence of sample

A : DPPH solution absorbance in the presence of sample

A_{Test} : Absorbance of sample in the absence of DPPH solution

From the graph of inhibition percentage, the extract concentration that causes 50% inhibition (IC50) is calculated.

2.6 β-Carotene Bleaching Test

β-carotene bleaching experiment is a well-known and frequently employed method for determining antioxidant capacity in substances and mixtures[19]. By comparing two competing chemical reactions, this activity is measured, wherein b-carotene, and/or model antioxidant has a role[20]. Thermic capacitance, more precisely 40°C, was used to dissolve β-carotene (2 mg) in chloroform (10 mL). The solution of β-carotene was then combined with linoleic acid (20 mg) and the emulsifier Tween80 (200 mg). At 40°C the removal of the chloroform from the ultimate result was followed and then distilled water (100 mL) was added to the mixture with continuous agitation. A volume of this mixture (0.2 mL) has been distributed to various test tubes with the sample solution. In a water bath (50°C) tubes were incubated with continual swaying for 2 hrs. The very first absorbance of extracts was evaluated (T_0) following the addition of the emulsion, and after 2 hrs, we repeat the process. (T_1), both at a wavelength of 470 nm. As a reference, BHA was used. All of the tests were performed three times.

The following formula was used to compute the lineolate/β-carotene radical's inhibition:

% of inhibition

$$100 - [\left(\frac{(\text{initial}(\beta\text{-carotene})(T0) - (\beta\text{-carotene})(T1))}{initial(\beta - \text{carotene})(T0)} \right) \times 100]$$

2.7 Peripheral Glucose Consumption

The diaphragm's glucose uptake was measured using rat diaphragm samples from animals that had been starved for 36 hrs before being sacrificed under a light ether anesthetic. The method established by Vallance-Owen *et al.*,[21] was used to test glucose consumption in muscle using one hemidiaphragm. In brief, the diaphragm was cut open, the bulky back part was eliminated, and the diaphragm was split into two equal parts that were placed into a flask containing a saline solution without glucose to clean all trash from the muscle. The saline solution's composition in g/L is 0.2 KCl, 0.2 $CaCl_2$, 8 NaCl, 1 $NaHCO_3$, 0.1 $MgCl_2$, 0.05 NaH_2PO_4. In the absence as well as in the presence of insulin (4UI /mL), the hemidiaphragms were incubated in a saline solution containing glucose (0.01 g/10ml) and an aqueous extract of *Lavandula stoechas* (5 mg /mL). The flasks were then oxygenated at 37°C with a combination of 5% of CO_2 and 95% of O_2, for 1 hr at a shaking rate of 90 cycles/min. The flasks were quickly cooled after the incubation, and the hemidiaphragms were removed, cleaned, blotted with filter paper, and weighed before (wet weight) and after 1 hr of oven drying (dry weight). By subtracting the glucose level after incubation from the glucose level prior to incubation, the results were reported as glucose consumed (mg/g of dry hemidiaphragm). Dry weight was calculated, and glucose was calculated spectrophotometrically using the glucose oxidase method.

2.8 Hemoglobin Glycosylation Test

To demonstrate *Lavandula stoechas's* antiglycation activity we followed the method established by Chauhan[22]. Various doses of the plant (100, 200, 400, 800, and then 1000 µg/mL) were combined in test tubes with 5µl of gentamycin and 1 ml of the hemoglobin solution. After 20 mins of incubation we add 1 mL of 20 mM Glucose in 10 mM phosphate buffer with pH: 4.7 to initiate the reaction. Then the tubes were incubated for 72 hrs at ambient temperature. Finally, to calculate the percentage of hemoglobin glycosylation, a spectrophotometer was set to 443 nm. Gallic acid is used as a standard, with different concentrations (100, 200, 400, 800, and 1000 µg/ mL) following the same previous protocol.

2.9 Statistical Analysis

Graph Pad Prism 5 was used to analyze the results, this was represented as the mean ± the Standard Deviation of the mean (SD). The findings were analyzed using one-way ANOVA.

3. Results

3.1 Determination of Total Phenolic Content and Antioxidant Effect

The determination of the polyphenolic composition by the Folin and Ciocalteu technique demonstrated that the average content of AqLs in phenol was equal to 146.71 ± 0.53 mg GAE/mg of AqLs. As regards the flavonoids, the quantity obtained was equal to 721.21 ± 0.21 µg QE /mg of AqLs.

First, the antioxidant potential of the aqueous extract of *L. stoechas* was measured using DPPH scavenging test

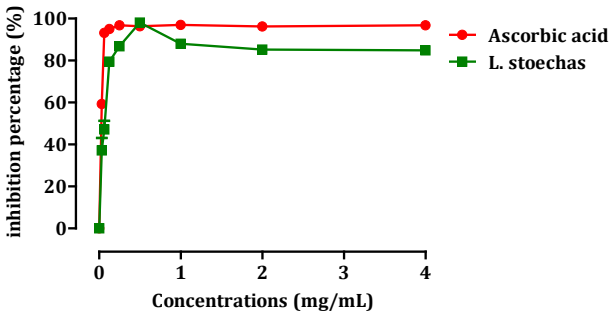

Figure 1. DPPH scavenging action (%) of different concentrations (mg/ mL) of the Ascorbic acid and *L. stoechas*. Values are means ± SEM.

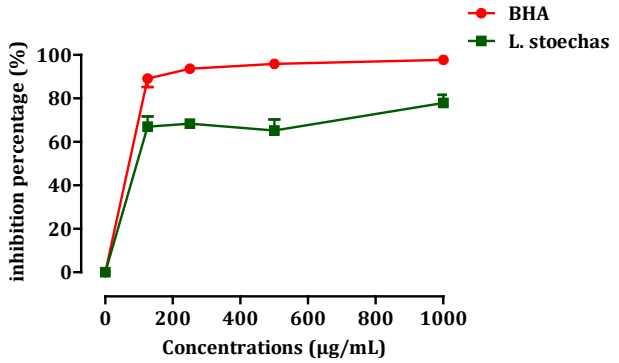

Figure 2. β-Carotene Bleaching Assay of different doses (µg/ml) of the aqueous extract of *L. stoechas* and BHA. Values are means ± SEM.

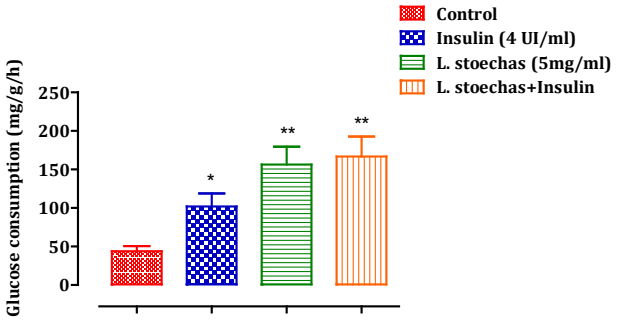

Figure 3. Separated normal rat hemidiaphragm glucose consumption in the presence and absence of insulin. Values are mean ± SEM.

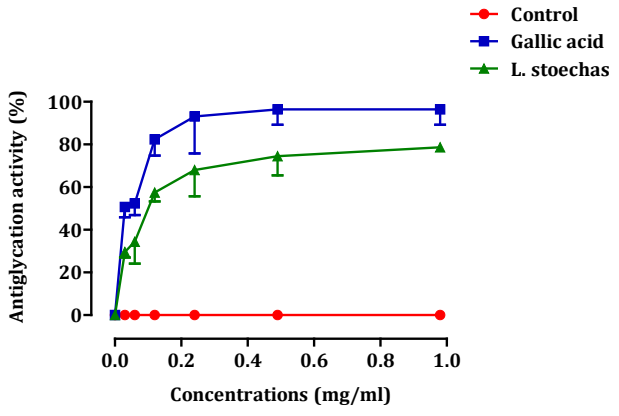

Figure 4. Hemoglobin glycosylation inhibition *in vitro* of the aqueous extract of *L. stoechas* and gallic acid. Values are mean ± SEM.

in a dose-dependent way. The extract was demonstrated in (Figure 1) — an important inhibitory action (IC50 = 0.031 ± 0.02mg / mL) against the free radical DPPH, which is approximately identical to that of the ascorbic acid (0.026 ± 0.001mg / mL).

On the second hand, in a concentration-dependent manner, the β-carotene bleaching assay of *L. stoechas* extract revealed enhanced inhibition (Figure 2). The extract had an important inhibitory activity so that the inhibitory concentration half-maximal (IC50) of the plant's extract and BHA were respectively 94.33 ± 12.5 µg/ml and 70.03 ± 5.77 µg/ml.

1.2 Peripheral Glucose Consumption

Glucose uptake by isolated rat hemidiaphragms results appears to indicate in (Figure 3) that when insulin is present at (4 UI/ mL), the glucose consumed is equal to 102.03± 15.36 mg/g/h, which is higher than the quantity consumed by the control group 43.91± 6.06 mg/g/h. When the aqueous extract of *L. stoechas* is present, the quantity of glucose uptake by isolated rat hemidiaphragms equals 156.38 ± 21.12 mg/g/h, so it increased in comparison to the control group. Furthermore, combining insulin (4 UI /mL) with an aqueous extract of *L. stoechas* enhances glucose consumption more than just insulin with 166.89 ± 23.56 mg/g/h.

1.3 Hemoglobin Glycosylation Test

Over a 72 hrs timeframe, the hemoglobin glycosylation inhibition *in vitro* test revealed in (Figure 4) a significant reduction of glycosylation in a concentration-dependent way. *L. stoechas* aqueous extract exhibited an important antiglycation activity with 48.94 ± 3.67 mg/mL, compared to the gallic acid, which had a stronger antiglycation efficacy than the plant extract with a value equal to 67.35 ± 3.85 mg/mL.

4. Discussion

Despite the massive lot of research on the chemical composition of *L. stoechas*, the vast bulk of these investigations focused on the plant's essential oil component[23]. In this study, we focused on the aqueous extract of *L. stoechas* which was considered the traditional method of treating diabetes. Various studies have shown that oxidative stress is involved in some way, either indirectly or directly role in the etiology of a number of illnesses, including diabetes[24]. Persistent hyperglycemia can cause tissues to produce an excessive amount of Reactive Oxygen Species (ROS). The high contents of antioxidants compounds decrease the negative effect of free radicals and protect against the damage induced by ROS[25]. In the current study, the aqueous extract of *L. stoechas* exhibited an important inhibition action versus the free radical. According to the results of a prior study, the *L. stoechas* methanolic extract had an important IC50 value, as shown by the DPPH assay for free radical scavenging activity[26]. Consequently, the antioxidant activity of the methanolic extract was significantly higher than the aqueous one[27]. Another research showed that the plant's ethanol extract had greater DPPH scavenging action than its water extract, the difference was statistically significant ($p<0,05$)[28]. On the other hand, the antioxidant activity of this extract was also assessed using the b-carotene bleaching technique, which revealed an enhanced inhibition, so the extract has an important inhibitory activity. Secondary metabolites produced by traditional medicinal plants have a wide range of pharmacological effects, including, antioxidant capability[29]. The Folin–Ciocalteu approach is a widely used method to determine the phenolic content of plant extracts in a quick and accurate manner[27]. Meanwhile, due to the strong reactivity of polyphenols as electrons or hydrogen donors and their ability to chelate metal ions, they exhibit the optimal chemistry for antioxidant action[30]. Therefore, according to the results of this study, the polyphenolic composition of *L. stoechas* aqueous extract by the Folin and Ciocalteu technique was important. As per the findings of a prior study, *L. stoechas* hydro-ethanolic extract showed an important value of gallic acid/mg of the extract[31]. This reveals that *L. stoechas* hydro-ethanolic extract contains almost the same quantity of polyphenols contained in the aqueous one. Based on our results, the quantity of flavonoids obtained is higher than the ethanolic extract of *Lavandula stoechas* according to another study[30]. The number of phenolics and flavonoid chemicals in the extracts and also the polarity variations between the extraction solvents can explain the wide range of results[32]. Furthermore, ethanol and water are less harmful than acetone, methanol, and other organic solvents from a toxicological standpoint[33]. The identification of 12 phytochemicals in the aqueous extract of *L. stoechas* was facilitated by the HPLC-ESI/MS research; 5-nonadecylresorcinol, Caffeic acid, 6"-O-Acetylgenistin, Quercetin 3-O-glucoside, Luteolin-O-glucuronide, Luteolin-O-glucoside, Rosmarinic

acid isomer, Apigenin-O-glucuronide, Rosmarinic acid, Salvianolic acid derivative, and Salvianolic acid B (Lithospermic acid B)[34].

Glycation is a non-enzymatic interaction among glucose and a free amino group of proteins that produces Advanced Glycation End-products (AGEs)[35]. The biomolecular harm in diabetes mellitus is caused by protein glycation, AGEs, and a rise in free radical action[36]. The hemoglobin glycation index can detect individuals whose hemoglobin concentrations are above or below normal in comparison to other individuals with the same glycemic level[37]. Since AGEs are abundant and oxidative stress is obtained from them in a number of illnesses and consequences related to diabetes. Finding and creating AGE inhibitors that can reduce their formation is of tremendous interest[38]. In comparison to gallic acid, the *in vitro* hemoglobin glycosylation inhibition experiment revealed significant glycosylation suppression over a 72 hr period; the aqueous extract of *L. stoechas* exhibited an important antiglycation activity. Hence, the aqueous extract of *L. stoechas* has a strong anti-diabetic impact.

The glucose intake by isolated rat hemidiaphragms in the presence of an aqueous extract of *L. stoechas* was also examined in this study. The insulin-like effect could explain the high level of glucose consumed by the rat's hemidiaphragm in the presence of the extract[39]. These data imply that the extract has active components that increase the impact of insulin at this concentration when paired with it. As a result, an elevation in glucose peripheral intake could be a mechanism involved in the current study's antihyperglycemic effect[40].

5. Conclusion

The existence of bioactive components such as polyphenols and flavonoids, which have antioxidant properties, was discovered in the aerial part of the Moroccan *Lavandula stoechas* in this study. Therefore, the antioxidant activity was approved based on the results of two *in vitro* experiments (β-carotene and DPPH). Moreover, the antidiabetic effect of the extract was indicated by the *in vitro* glycosylation of hemoglobin and also by the *in vitro* glucose absorption research by the hemidiaphragm of isolated rats. This research was done to improve the plant's anti-diabetic properties with the intention of using it as a supplementary food to treat diabetes and its complications.

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7. References

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