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ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF ETHANOLIC EXTRACT OF *LAVANDULA STOECHAS* L. FROM TAOUNATE REGION IN MOROCCO

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ABSTRACT

The extract obtained from the *Lavandula stoechas* (Lamiaceae) is used in Moroccan folk medicine as remedies for the treatment of various inflammatory diseases. This study aims to investigate *in vitro* antioxidant activities of *L. stoechas* and to examine the *in vivo*–anti-inflammatory effect of the mixture ethanolic/water extract (4/1.V/V) of aerial parts of *Lavandula stoechas.* The antioxidant power of the ethanolic extract of *L. stoechas* was evaluated by using, 2,2-diphenyl-1-picryl hydrazyl (DPPH) and phosphomolybdenum assay *in vitro* methods. The anti-inflammatory activity was evaluated by Carrageenan-Induced Rat Paw Edema method. In DPPH scavenging assay the IC50 value of the extract was found to be 1.2µg/ml while the IC50 value of the reference standard Butylated hydroxytoluene (BHT) was 0.2µg/ml. The antioxidant capacity of *L. stoechas* extract showed 255,5 µg/ml of equivalent to ascorbic acid in comparison with gallic acid which is 155µg/ml equivalent to ascorbic acid, it also demonstrated that antioxidant power increase in a dose dependent manner. The ethanolic extracts of *L. stoechas* (1000 and 2000 mg/kg, body weight "b.w") inhibited the inflammation induced by carrageenan in rats in a dose dependent manner. At dose of 2000 mg/kg, b.w, *L. stoechas* produced a significant inhibition of inflammation at 74 % compared to 69 % for diclofenac at 1 %. This study suggest that *Lavandula stoechas* may act as a chemopreventive agent, providing antioxidant properties and offering effective protection from free radicals, and confirm the Moroccan traditional use of this plant for the treatment of inflammatory diseases. Then, it's necessary to identify and isolate the compounds that are responsible to the antioxidant and anti-inflammatory effects.

Key words: Anti-inflammatory activity, Antioxidant activity, DPPH, *Lavandula stoechas* L, Phosphomolybdenum complex, Sonication.

INTRODUCTION

Taounate is the widest Northern Province of Morocco with a distance of 5616 km2, which is covered by three bioclimatic strata that promote the development of several species of medicinal and aromatic plants. The genus "Lavandula" (Lamiaceae) includes over 34 species, self-sown in the Mediterranean basin (Miller, 1985). *Lavandula angustifolia* Mill, (Lavender), *Lavandula latifolia* Medik, *Lavandula fragrans* L, *Lavandula stoechas* L. and *Lavandula multifida* L. are the most used in traditional medicine (Gamez *et al*., 1987) and in pharmaceutical and cosmetic industries (Cavanagh *et al*., 2002). *L. stoechas* is widely distributed in Mediterranean region. This plant is used traditionally in Taounate to treat rheumatism and digestive system (EL-hilaly *et al*., 2003).

Oxidative stress has been linked to various diseases (Halliwell, 1994) while food-industry has been long concerned with other issues, such as; rancidity and oxidative spoilage of food stuff (Shahidi *et al*., 1992). Antioxidants are often added to food in order to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation of step leading to the termination of the reaction and a delay in the oxidation process. However, the commonly synthetic antioxidants

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used such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens (Imaida *et al*., 1983; Madhavi *et al.*, 1996). Thus, antioxidants interested both areas: food scientists and health professionals, and there has been a convergence of interest among researchers in these fields to the role of antioxidants in the diet and their impact on human health has become a priority.

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. There are a lot of treatments available for inflammation, but is not yet satisfying the needs of patients who are suffering from inflammatory diseases. Recently there is a vast prevalence of this disease due to the continuous change in the life style of people. Hence, it is necessary to introduce such therapy which would be more effective and more reliable, since many drugs from plant origin are used through a centuries for treatment of human diseases and also for the food needs.

This study is conducting to verify antioxidant and anti-inflammatory activities of this species. For this aim, the powder of *L. steachas* aerial parts was extracted with 75% of aqueous ethanol after a preliminary extraction with hexan, and the extracts were screened for antioxidant activities using two *in vitro* assay models, the DPPH radical scavenging assay and the phosphomolybdenum method. We also evaluate the ability of extracts to inhibit the paw edema in rats induced by carrageenan. Their anti-inflammatory activity was evaluated in comparison to that of the non-steroidal anti-inflammatory drug such Diclofenac at 1%.

MATERIALS AND METHODS

Plant collection

The aerial parts of *L. stoechas* are collected in the province of Taounate in Morocco, between April and June 2012. Then they are air-dried at 40° C with forced ventilation for two days. The Botanical identification and the Authenticated voucher specimens deposited in the Herbarium of The National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

The Ultrasound-assisted extraction (US)

Twenty grams of flour of *L. stoechas* are mixed with 150 mL of n-hexane at 35 kHz frequency during 45 min, and with a temperature lower than 25 °C. After being extracted, the mixture filtered under vacuum through Whatman paper, and the solvent removed. Then the plant's material re-extracted again with a mixture of ethanol /water (4:1 (v/v)) for 45 min under the same conditions. The final extract was recuperated from the mixture (ethanol/water) after filtration by Watman paper and evaporation under vacuum at 40°C on a rotary evaporator.

Phytochemical screening

The ethanol/water extract is screened for phytochemical constituents (Coumarins, Leucoanthocyans, Flavonoids, Mucilags, Tannins, Sterols and Terpens, Quinones and Cardiac glycosids) using a simple qualitative methods as described in the study of (Paris and Nothis, 1996; Diallo, 2005) . The extract is concentrated and it was dried under low pressure. Then, the appearance color of the extracts was noted.

Determination of total flavonoids content

The flavonoid content of extract of the *L. steochas* is calculated by the method described by (Ordoněz *et al.*, 2006). This method involves adding 0.5 mL of AlCl3 solution (2% in absolute ethanol) to 0.5 mL of sample. After one hour of incubation at a temperature room, the absorbance is measured at 420 nm, and then the total flavonoid content is calculated in terms of equivalent Quercetin as a reference to the standard curve.

Antioxidant tests

Although there is no standardized method to evaluate the antioxidant potential of foods and biological systems, it is recommended to evaluate the antioxidant activity by different methods (Frankel *et al*., 2000).

Determination of free radical scavenging activity by DPPH method

The ability of the *L. stoechas* extracts to scavenge DPPH radicals was determined according to the procedure described by (Soler-Rivas *et al*., 2000) with slight modifications. Briefly, 100 µl of various concentrations of the extract was added to 10 ml of a methanol/DPPH solution (1.01×10^{-2} M). The mixture was vigorously shaken and then allowed to stand at a room temperature for 30 min in the dark. The absorbance of the mixture measured at 517 nm by using a double-beam UV–vis Camspec M550 spectrophotometer. A mixture of 100 µl of methanol and 10 ml of DPPH solution is used as control. The scavenging activity was expressed as percentage of inhibition using the following equation (Blois, 1958):

$$\%\text{Inhibition}=\left\{\frac{(AB-AS)}{AB}\right\}\times 100$$

Where A_B is the absorbance of the control reaction (containing all reagents except the extract), and A_S is the absorbance of the extract.BHT used as positive control. All the tests are carried out in triplicate. The extract concentration providing 50% of inhibition (IC50)

is calculated from the graph of inhibition percentage plotted against extract concentrations (4.0, 2.0, 1.0, 0.5 and 0.25 g/L).

Evaluation of total antioxidant capacity by Phosphomolybdenum method

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described by (Prieto *et al.*, 1999). The tubes containing the reaction solution were capped and incubated in boiling water at 95°C for 90 min. The control consisted on 0.3 mL of methanol the absorbance of the solution is measured at 695 nm using a spectrophotometer. The antioxidant capacity of each sample expressed as ascorbic acid (A.A) equivalent using the following linear equation established using ascorbic acid as standard:

[$A = 0.0037C + 0.0343$; $R^2 = 0.991$].

Where:

A: is the absorbance at 695 nm,

C: is the concentration as ascorbic acid equivalent (µg/ml).

The values are presented as the means of triplicate analysis.

Anti-inflammatory activity

Carrageenan-Induced Rat Paw Edema: Acute inflammation in the rats produced according to the method described by (Winter *et al*., 1962). Therefore, four groups of five rats were used for the study. Group 1 served as the control group receiving normal saline, animals in group 2 were given topical application of Diclofenac gel while groups 3 and 4 were given topical application of cream formulated in our laboratory by mixing the neutral cream with extract at doses of 1000 and 2000 mg/kg, b.w.

The cream was applied 90 min before the induction of inflammation. The percentage of inflammation inhibition was calculated by the method previously described. The efficiency of cream was evaluated in comparison with Diclofenac at 1 %. The percentage inhibition was calculated thus:

$$\%\ \text{inhibition} = \left\{ \frac{\left((St-S0)control - (St-So)treated \right)}{(St-S0)control} \right\} \times 100$$

Where:

S_t = the mean paw size for each group after the carrageenan treatment.

And

S_o = the mean paw size obtained for each group before the carrageenan treatment.

Statistical Analysis

Data were expressed as Mean ± SEM. Comparisons of means were performed by using the t-test of Student. The level of statistical significance was set at $p < 0.05$.

RESULTS

Phytochemical screening

As shown in Table 1, phytochemical screening of extract of *L. stoechas* revealed a presence of Tannins, Catechic Tannins, Flavonoids, Sterols, Coumarins, Quinones, Leucoanthocyans and Mucilages compounds . However, Gallic tannins and Quinones were not detected.

Determination of the content of total flavonoids

The total flavonoid content of the ethanolic extract of *lavandula stoechas* is 39,5 µg expressed as Quercetin equivalent in micrograms per mg of extract with a proportion of 3, 95 % of dry matter.

Determination of free radical scavenging activity by DPPH method

The DPPH radical scavenging is a sensitive antioxidant assay and is independent of substrate polarity (Yamaguchi *et al*., 1998), this model is widely used to evaluate antioxidant activities in a relatively short time compared with other methods. The antioxidant activity of ethanol extract of *L. stoechas* is tested with DPPH scavenging assay. At a concentration of 4, 2 and 1g/l, the ethanolic extract decreased under the present experimental condition, by 85.5, 57.8 and 44% (% the DPPH signal) as compared to a 78.3, 73, and 65% decrease with BHT. The IC50 found in this study for the extract of LS were high as 1, 4 g/L, as compared to 0,2g/L for BHT (Figure2).

Evaluation of total antioxidant capacity by Phosphomolybdenum method

The antioxidant capacity of *L. stoechas* extract showed 255,5 µg/ml of equivalent of ascorbic acid in comparison to gallic acid 155 µg/ml equivalent to ascorbic acid at 100 µg/ml concentration, it also found to increase in a dose dependent manner (Figure 3).

Anti-inflammatory activity

Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3 h. At a dose of 1000 mg/kg *L. stoechas* extract produced a maximum inhibition of carrageenan induced inflammation of 44±8.7%. We noted that *L. stoechas* extract at a dose of 2000 mg/kg, b.w., produced a maximum inhibition of carrageenan induced inflammation of 74±7 %, mainly at 5 h after treatment compared to Diclofenac which produced an inhibition of 69,5±10.3% and the inflammatory effect was not longer after 4 hours (Figure 4).

Table 1. Phytochemical screening of ethanolic extract of *Lavandula Steochas* L.

Phytochemical	Ethanolic extract
Tannins	+
Catechic tannin	+
Gallic tannin	-
Flavonoids	+
Sterols and terpenes	+
Coumarins	+
Quinones	-
Leucoanthocyans	+
Cardiac glycosides	+
Mucilages	+

Presence of chemical compounds is: (+) = important; (-) = absent

Fig 1. Standard curve of Quercetin

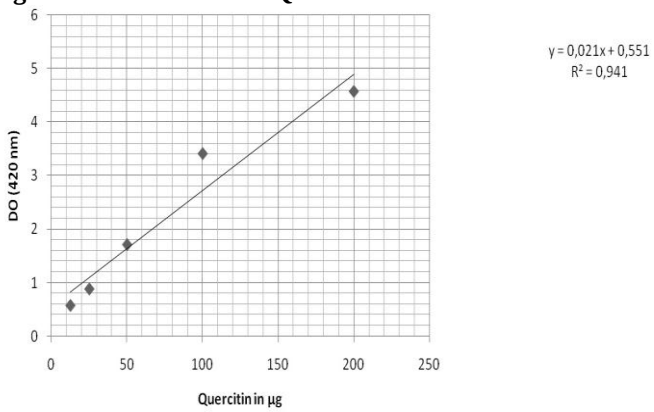


Fig 2. Antioxidant activity of ethanolic extract of *L. stoechas* by DPPH method

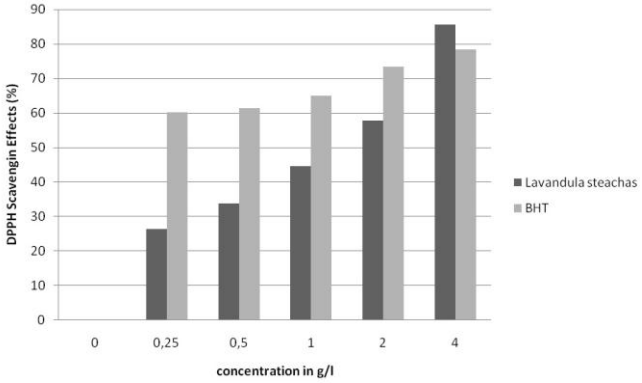


Fig 3. Antioxidant capacity of extract of *L. stoechas* by phosphomolybdenum method

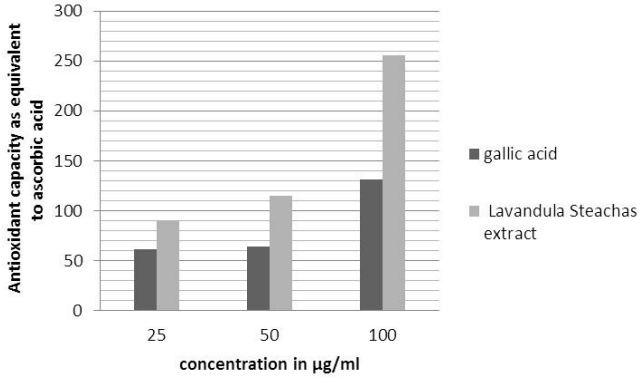
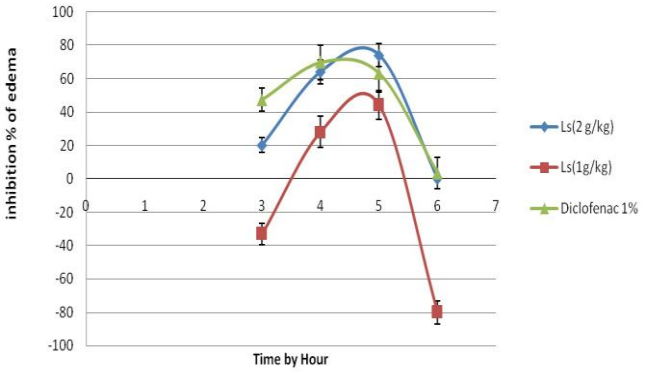


Fig 4. Anti-inflammatory effect of *L. Stoechas* compared to Diclofenac. N= 24



DISCUSSION

It is known that antioxidant compound in ethanol extract have the free radical scavenging ability due to the presence of flavonoids and tannins in the plants which are probably responsible for the free radical scavenging effect (Polterai, 1997).

The anti-inflammatory activity of *L. stoechas* is due to their richness in flavonoids, tannins and mucilages which has been proven by several studies (González-Gallego *et al*., 2007; García-Lafuente *et al.*, 2009; Sindhu *et al*., 2012). Previous studies demonstrated also the role of flavonoids in anti-inflammatory process (Middleton *et al*., 2000; Chi *et al*., 2001; Havsteen, 2002). The early phase, 2 h after carrageenan injection, is attributed to the release of histamine and serotonin followed by a later phase of edema due to production of bradykinin and prostaglandins. This later phase has been reported to be sensitive to both steroidal and non-steroidal anti-inflammatory agents. In this study, the extracts did not show any significant anti-inflammatory effect in the early

phase but showed significant effect at the later phases after 4-5 h. The results suggest that the extract acts at the later phase involving arachidonic acid metabolites probably by the inhibition of cyclooxygenases (Ayoola et al., 2009).

CONCLUSION

This study demonstrated the presence of bioactive principles in the leaves and flowers of Moroccan *Lavandula stoechas*: flavonoids, tannins, sterols and mucilages which possess antioxidant and anti-inflammatory properties. Ethanolic extract of *L. stoechas* seems to present a real interest and potential for their antioxidant activities that were established by two *in vitro* tests (DPPH and PPM). The evaluation of anti-inflammatory power shows that this extract possesses a good anti-inflammatory activity; this might be due to the richness of the ethanolic extract of *L.stoechas* in bioactive compounds, mainly flavonoids. All these results confirm the use of these plants in inflammatory diseases in traditional Moroccan medicine. It will be useful to further investigations to identify the molecules responsible for the anti-inflammatory activity.

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