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Full Length Research Paper

Antioxidant activity of the essential oil from the flowers of *Lavandula stoechas*

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This study was aimed at determining the chemical constituents and evaluating the antioxidant activity of essential oil of dry flowers' of lavender (*Lavandula stoechas*). Extraction of essential oil was carried out by water distillation. The essential oils yield based on dry weight was 3.41% (v/w). The gas chromatography with flame ionization detector (GC/FID) technique was used and identification of separated components was achieved by retention times in comparison with respective standards. According to the results, 6 compounds were identified and the most important ones are: linalyl acetate (15.26%), linalool (10.68%), 1-8 cineole (10.25%), γ-terpinene (11.2%) and camphor (11.25%). The study of antioxidant power of this oils was carried out by 1,1-diphenyl-2-picryhydrazyl (DPPH•) method. The results obtained showed the existence of an antioxidant activity of the essential oil from the dried flowers of *L. stoechas*, but less effective compared with vitamin E.

Key words: Essential oil, *Lavandula stoechas* composition, antioxidant activity.

INTRODUCTION

In recent years, there has been a growing interest in the use of antioxidant plants for scientific research as well as industrial (pharmaceutical and cosmetic) purposes. The volatile chemicals and extracts of some plants have been studied for theirs antioxidant activities because they inhibit oxidative damage and may consequently prevent inflammatory conditions (Repetto and Lisiuy, 2002) in many domains, including medicine, nutrition and cosmetics (Djeridane et al., 2006).

In Algeria, a wide range of medicinal plants are used in folk medicine for the treatment of different diseases (Allali et al., 2008). In the present study, one of the most used plants in Algeria, *Lavandula stoechas*, was investigated. *L. stoechas* is a plant of the Lamiaceae family that is widely used in folk medicine in different parts of the world. The flowers' extracts are reputed to possess antibacterial, antifungal and antioxidant properties (Laib, 2011). This led us to study the chemical composition and the antioxidant activity of the essential oil of the dry flowers of the lavender.

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MATERIALS AND METHODS

Sampling

The flowers of *L. stoechas* were collected from Institute of Food, Nutrition and Agroalimentary Technologies (I.N.A.T.A.A), University Mentouri Constantine, Algeria, 7 km away from the town center. The harvest was undertaken manually in June 2010, when it is at flowering stage.

Process of extraction

The extraction of the essential oil of the dried flowers of *L. stoechas* was made by a water distillation for about 3 h. The essential oils were stored in a sealed glass vial in a refrigerator at 4°C until required.

Determination of the chemical composition of essential oil by gas chromatography with flame ionization detector (GC/FID)

The analytical study of the essential oil of *L. stoechas* was carried out by the GL1/K Complex Department ADM/Soc Skikda Laboratory Service, with GC type VARIAN CHROMPACK-CP 3800 per injection of 0.2 µL of essential oil using a microphone-syringe. The carrier gas was helium (He) of a flow of 0.3 ml/min. The column used is a capillary tube of type CP-Chirasil-Dex CB fused silica wall

Table 1. Concentration in % and retention time of the various compounds obtained by GC/FID analysis of the essential oil of *Lavandula stoechas.*

Compound	Retention time (min)	% (w/w)
Camphor	22.365	**11.25**
1,4-Cineole	28.210	0.35
γ-terpinene	52.339	**11.20**
1,8-cineole	53.064	**10.25**
Linalool	86.970	**10.68**
Linalyl acetate	88.390	**15.26**

coated open tubular (WCOT), 25 m length and 0.25 mm of internal diameter. The thickness of the stationary phase is 0.25 µM; the programming of the temperature of the initial column of injection is 70°C during 2.50 min, then rises by stage of 15ºC/ min with 240°C during 20 min; the detector used for this analysis is type FID with a temperature of 250°C. For the calibration curves of each compound, 6 standard solutions in hexane (α-pinene, cineol, camphor, linalool, linalyl acetate and γ-terpinene) were used as data points. The standards are from Aldrich-Chemie Company.

Evaluation of the antioxidant activity

The antioxidant activity *in vitro* was evaluated by the measurement of the capacity of trapping of radical 1,1-diphenyl-2-picryhydrazyl (DPPH•), according to the method described by Burits and Bucar (2000), where 50 µL of each methanolic solution of oil essential is tested with various concentrations (200, 400, 600, 800 and 1000 µg/ml) that are mixed with 5 ml of a methanolic solution of DPPH (0.004%). After one incubation period of 30 min at room temperature, the absorbance was read at 517 nm. The inhibition of free radical DPPH by vitamin E was also analyzed with the same concentration for comparison. The kinetics of the reaction and the parameters of calculation of the antioxidant activity for the vitamin E and essential oil (percentage of inhibition, index IC_{50}, the time of balance TC50 and the effectiveness of antiradical (EA) was also detected. All the tests are carried out three times.

Determination of the inhibition percentage

According to Sharififar et al. (2007), the inhibition of the free radical of DPPH expressed as a percentage (%I) is calculated as follows:

$$I\ (\%) = (A_C - A_S) / A_C \times 100$$

Where A_C is the absorbance of control (containing all the reagents except the test sample) and A_S is the absorbance of the sample.

The kinetics of the reactions of essential oil and the vitamin E with the DPPH• is registered with each examined concentration. The concentrations of essential oil and vitamin E in function to the percentages inhibited of $DPPH^{\bullet}$, are traced at the end of the reaction in order to obtain index IC_{50}. This parameter is defined as the necessary antioxidant concentration to decrease the initial concentration of the $DPPH^{\bullet}$ by 50% (Sharififar et al., 2007).

Determination of T_{EC50}

The parameter T_{EC50} is defined as the time needed to attain a balance with an antioxidant concentration equal to IC_{50}. This time is calculated graphically (Sharififar et al., 2007).

Determination of the antiradical effectiveness (AE)

Two factors, IC_{50} and T_{E50}, are combined in order to obtain the antiradical effectiveness (AE) parameter (Sharififar et al., 2007). This is calculated as:

$$AE = 1/IC_{50} \times T_{EC50}$$

RESULTS AND DISCUSSION

Moisture content

Determination of flowers' moisture of *L. stoechas* revealed a rate equal to half of the fresh flowers weight. This rate corresponded to approximately 55.40%. This means that 44.60% represent the dry material rate used for the extraction of essential oils.

The yield of essential oil

We recall that essential oil is extracted from the dry flowers of *L. stoechas*. Our results indicated that a total yield of 3.41% was obtained.

Principal compounds of essential oil detected by GC/FID

The analysis of the essential oil of *L. stoechas* by gas chromatography made it possible to identify 49 terpenic compounds as shown in Table 1, per order of elution. Six components representing the sum of the percentages of the components obtained were identified, of which 67.29% are oxygenated monoterpene derivatives and 15.3% are hydrocarbons monoterpene. It seems that the components of the essential oil of *L. stoechas* are monoterpenes. The major components of this oil are: linalyl acetate (15.26%), linalool (10.68%), 1,8-cineol (10.25%), γ-terpinene (11.2%) and camphor (11.25%). However, these results are different from those indicated by some authors. For example, Kulevanova' et al. (2000) analyzed the chemical composition of the essential oil of flowers of *Lavandula* collected from mountain of Kozjak

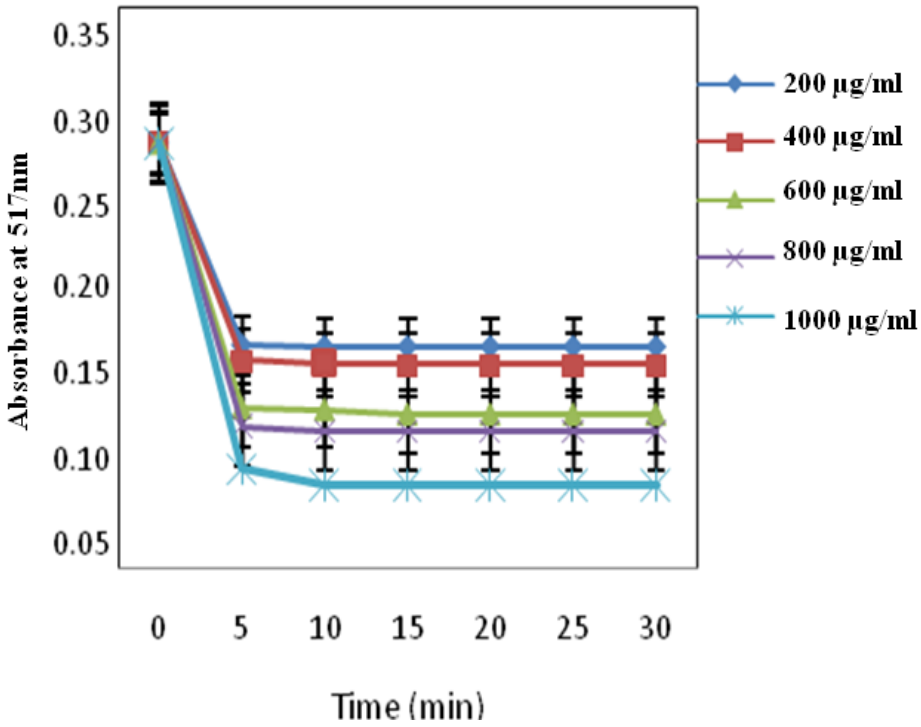

Figure 1. Kinetics of reduction of the DPPH with the vitamin E.

Macedonia, and find 32 components with a prevalence of linalool (25.7%), linalyl acetate (23.2%) and lavandulyl acetate (12.4%) with a predominance of the monoterpene components and the presence of sesquiterpene hydrocarbons and its oxygenated derivatives. Verma et al. (2009) have also studied the composition of oil essential of the flowers of *Lavandula officinalis* cultivated in Uttarakand (India); they identified 37 monoterpene compounds, the major compounds being linalyl acetate (47.56%), linalool (28.06%), lavandulyl acetate (4.34%) and α- terpineol (3.7%). Sun and Sun (2002) compared the chemical constituents of essential oils of *Lavandula* obtained by various methods of extraction. They found that the linalyl acetate (35.44%) and the linalool (18.70%) are prevalent in the essential oils obtained by steam distillation, while their values are respectively 2.63 and 4.04% in the case of solvent extraction; 36.80 and 43.47% in the case of extraction by microwave.

From these results, we noticed that the chemical composition of the essential oil of the species *L. stoechas* cultivated in Constantine was different from those obtained from many experiments on the same species, with a prevalence of the monoterpene compounds in the majority of the cases, but with different proportions. This difference in composition is probably due to various conditions, particularly the environment, the genotype, the geographic origin, the period of harvest, the place of drying, the temperature and the duration of drying, the parasites and the method of extraction (Belhadj et al., 2006).

Antioxidant activity

Kinetics of the reaction

The kinetics of reduction of free radical DPPH obtained for each concentration of vitamin E and essential oil is indicated in Figures 1 and 2. For the two compounds examined (vitamin E and essential oil), the reaction is biphasic, with a fast weakening in the absorbance in the first minutes, followed by a slower stage until balance is reached. At 5 min, the essential oil and vitamin E followed the same zone in different concentrations. After 5 min, vitamin E goes over the second zone while the essential oil stays in the first zone. During 10 min, essential oil at concentration 1000 µg/ml goes over the second zone, while at 15 min later all the concentrations of the second zone of essential oils remain stable. When we carried out the reaction between the DPPH and vitamin E donor of hydrogen, we noted that the reaction attained a balance at a short time compared to the essential oil of *L. stoechas.* The antioxidant activity is dependent on the mobility of the hydrogen atom of the hydroxyl group of the phenolic compounds of essential oil. In the presence of a free radical DPPH•, the H atom is transferred to the latter and then transformed into a stable molecule DPPH$^{\cdot}$. This causes a reduction in the concentration of the free radical

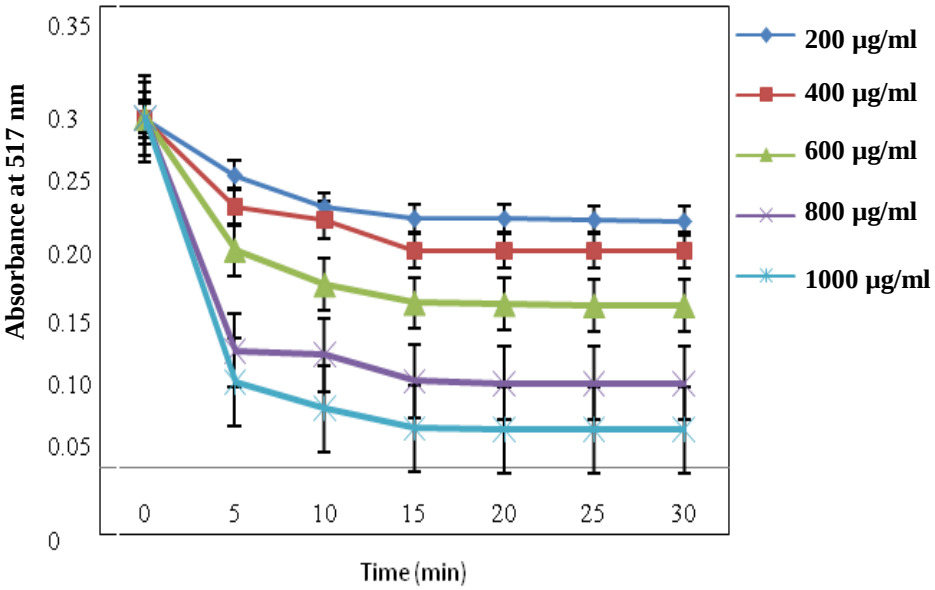

Figure 2. Kinetics of reduction of the DPPH with the essential oil of the lavender.

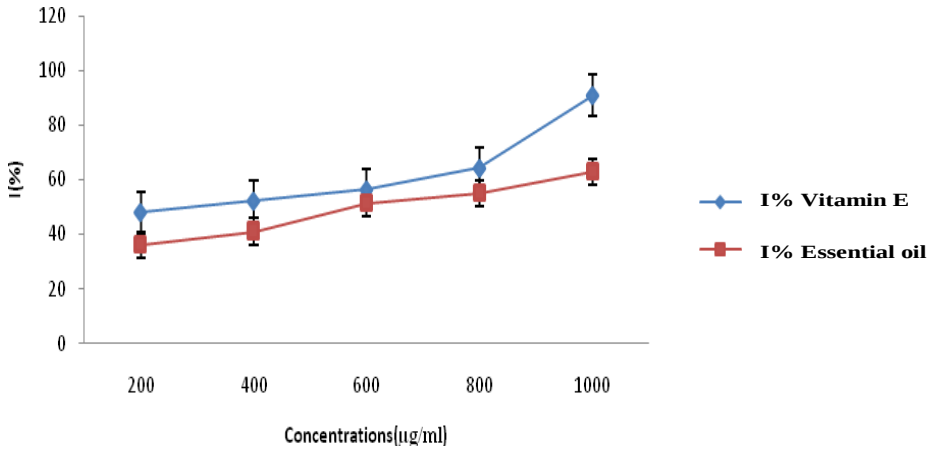

Figure 3. Inhibition percentage for essential oil and vitamin E.

radical and also the absorbance during the reaction time until the exhaustion of the capacity of antioxidant donor of hydrogen.

Inhibition percentage

The results obtained during the test of measurement of inhibition percentage of radical DPPH$^{\bullet}$ are shown in Figure 3. It seems that the inhibition percentage of the free radical increases with the increase in the concentration of the vitamin E or the essential oil of the lavender. It was also noticed that the inhibition percentage of the free radical for essential oil is lower than that of vitamin E for all the concentrations used. For a concentration of 1000 µg/ml, essential oil reveals an inhibition percentage of 63.01 ± 5.26, while vitamin E is inhibited with 90.94 ± 2.66 of DPPH.

Value of CI_{50}

The IC_{50} is conversely related to a compound antioxidant capacity because it expresses the necessary quantity of antioxidant and decreases the concentration of the free radical of 50%. The lower value of CI_{50} has the most important antioxidant activity (Figure 4). The essential oil of the lavender could bring back the stable free radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•) to the yellow-colored diphenylpicrylhydrazine (DPPH) with and IC_{50} of

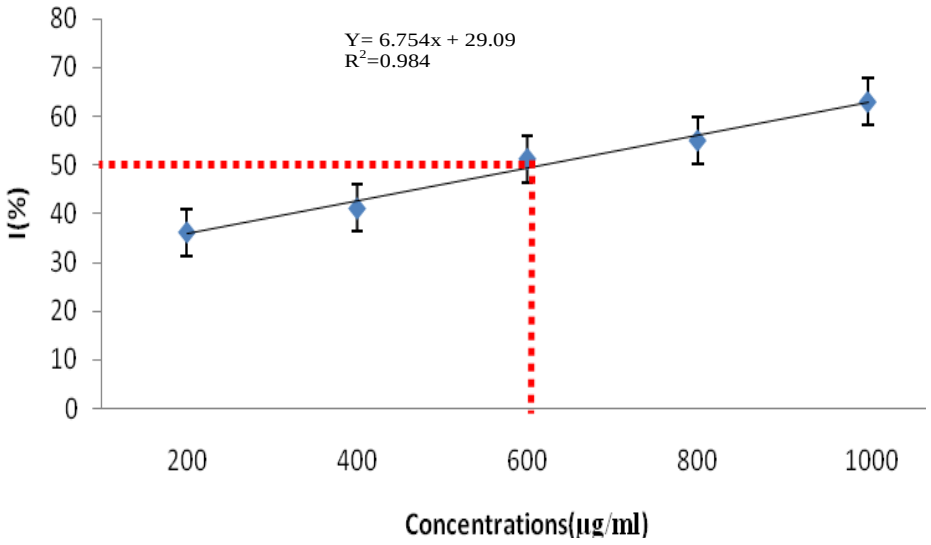

Figure 4. Calculation of IC_{50} for the lavender essential oil.

584 ± 0.58 µg/ml, showing an antioxidant activity lower than that of vitamin E. It seems from to these results, that vitamin E is the most effective antioxidant with an IC_{50} of 384 ± 0.76 µg/ml compared to the studied essential oil.

Value of T_{EC50}

We chose the state of balance (where it proves that the reaction does not progress further) as period of measurement. The time to attain the state of balance depends on the reactivity of antioxidants and the concentrations used. It is noted that vitamin E reacts in a faster way with DPPH•. The T_{EC50} for essential oil studied was of 17 ± 1 min, whereas vitamin E needed only 8 ± 0.66 min to decrease the concentration of the free radical by 50%.

Antiradical effectiveness

A new parameter defined as the antiradical effectiveness, combines two parameters (IC_{50} and T_{EC50}) in order to easily characterize the behavior of a substance as an antioxidant. It seems that the essential oil of *L. stoechas* has an antioxidant activity, but it is less effective than that of vitamin E. It also seems that this activity is related to the presence of the phenolic compounds in essential oil. The main role of these components as reducer of the free radicals has been previously reported (Villano et al., 2001). The camphor which is the major compound of our essential oil with a concentration of 11.25% has a strong antioxidant activity (Svoboda and Hampson, 1999). In fact, only the chief compounds of essential oil are responsible for this antioxidant activity. However, there can be also other less important compounds that can interact in a synergistic or antagonistic way to create an effective system with respect to the free radicals (Lu and Foo, 2001; Sing et al., 2006). The presence of carvacrol even with weak concentration in the essential oil of *L. stoechas* (0.9%) can explain the activity of trapping of radical DPPH.

Economou et al. (1991) has reported antioxidant activity of the essential oil from flowers of *Lavandula* kind against the oxidizing deterioration of the lard. In the same way, Hui et al. (2010) have analyzed antioxidant capacity of lavender essential oil on the inhibition of the peroxidation of linoleic acid and the inhibition of the peroxidation of linoleic acid by vitamin E with the same concentration for the comparison. They noted that the lavender essential oil shows an antioxidant activity stronger than vitamin E against the peroxidation of the lipids. In two studies carried out separately by Lis-Balchin and Deans (1997) and Lis-Balchin (2002), it was reported that there are no correlations between the percentage of the principal components, linalol and linalyl acetate and the antioxidant activity of essential oils extracted from lavender. These contradictory results are due probably to the difference of the chemical composition of these essential oils (Lis-Balchin, 2002).

Conclusion

The objective of this work was to analyze the chemical constituents and to evaluate the antioxidant activity of the essential oil of dried flowers of *L. stoechas*. The results obtained indicate that the yield of extraction of essential oil by water distillation was 1.36 ± 0.2%. The kinetics of extraction showed that the total essential oil was extracted at

the end of the first 80 min. Furthermore, the composition of essential oil was analyzed by GC/FID. It identifies six terpene compounds with GC/FID, of which the most principal compounds were: linalyl acetate (15.26%), Linalool (10.68%), 1-8cineole (10.25%), γ-terpinene (11.2%) and camphor (11.25%). Moreover, the essential oil has an antioxidant activity but it is less effective by comparison with vitamin E. In future studies, an analysis of the mechanism of the action of these compounds is recommended.

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