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Chemical Composition and Biological Potential of Different Extracts of Two Medicinal Plants from Northern Algeria

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Abstract: This work aime to study the chemical composition and the main biological properties contained in the extracts of *Lavandula stoechas* and *Pistacia lentiscus* collected in northern Algeria. *Lavandula stoechas* and *Pistacia lentiscus* are two medicinal plants belonging to the botanical family Labiatae and Anacardeacea respectively. They are grow wild in the northen Algeria and widely used in traditional Algerian medicine. The GC/MS analysis of the essential oils of *Lavandula stoechas* extracted by hydrodistillation showed that the latter are of fenchone chemotype whatever the period and the site of harvest. LC/MS analysis of tannin powders extracted by maceration of the leaves in a hydroalcoholic solvent reveals that they mainly contain rosmarinic acid. Essential oils extracted from the leaves of *Pistacia lentiscus* showed a large variability in terpenoid. The antioxidant activity of the different essential oils varied according to the harvesting period and the altitude. The highest antiradical effects were observed with the FRAP test. An another side the *Pistacia lentiscus* extracts showed to have an α-glucosidase inhibition effect that was more or less impactful according to the plant's living environment (mountain or littoral) and the considered organ (leaves, stem barks or fruits).

Keywords: *Lavandula stoechas*, *Pistacia lentiscus*, Essential oils, Antioxidant activity, α-glucosidase inhibition

Introduction

Remedies made from natural substances and plants have been widely used for the management of variable diseases due to their safety and reduced side effects.Today, doctors, health organizations and pharmaceutical laboratories recognize the value and effectiveness of treatments based on medicinal and aromatic plants. They admit that the place of these plants is increasingly important in modern pharmaceutical production. Furthermore, nearly 25% of drugs currently used in modern medicine are derived from medicinal plants (Kifle, 2021). Northern Algeria is characterized by a diverse plant cover and the genera Lavandula and Pistacia are the most

important medicinal and aromatic plants in this region. *Lavandula stoechas* from the *Lamiaceae* family has been used for centuries, in the form of essential oils or dried flowers, for numerous cosmetic and therapeutic applications. Traditionally, this plant is used as a carminative, antispasmodic, unclogging, stimulating, expectorant and against skin problems (Matos et al., 2009). *Pistacia lentiscus* (lentisk) is a plant species of the Anacardiaceae family. Lentisk has been used in traditional medicine for the treatment of several diseases, such as gastrointestinal diseases, eczema, throat infections (Dragovi´c et al.,2020; Ljubuncic et al., 2005), diabetes for its hypoglycemic effect (Jamila et al., 2014) tooth ache and respiratory disorders (Abdeldjelil, et al.,2014).

Previously, the chemical profile and antibacterial properties of essential oils of *Lavandula stoechas* and *Pistacia lentiscus* have mainly been examined (Alejandro et al., 2015; Boudieb et al., 2019). However, work on other significant biological activities of essential oils and hydroalcoholic extracts of these two Algerian medicinal plants is limited. This present work was developed to explore the chemical composition of the essential oils of *Lavandula stoechas* and *Pistacia lentiscus* and to study the effect of the harvest season on the variation of their chemotypes as well as on their antioxidant power. The other objective of this study is the characterization of the phytochemical profiles of leaves, stem bark and mastic fruits of lentiscus harvested at two different altitudes (mountain and coastal) with regard to their antidiabetic potential. This is carried out using a metabolomics approach based on the link between phytochemical profiling (evaluated by ultra-high performance liquid chromatography coupled with UHPLC-ESI-HRMS mass spectrometry) and the α-glucosidase assay.

Materials and Methods

Chemicals

The main chemicals used for the measurements of α-glucosidase inhibition were α-glucosidase from Bacillus stearothermophilus (G3651-250 UN, 84 units/mg, 86% protein), p-nitrophenyl α-D-glucopyranoside (N1377,≥99.0%) and acarbose (PHR1253-Pharmaceutical Secondary Standard; Certified Reference Material). The analytical standards for the UHPLC-ESI-HRMS analysis were (−)-epigallocatechin (EGC)(≥90%, 08108-supeclo), myricitrin (≥99.0%, 91255, Sigma-Aldrich, Saint Quentin Fallavier, France),apigenin 7-glucoside (≥97.0%, 44692, Supelco) and tiliroside (≥95.0%, PHL89809-phyproof®Reference Substance). These analytical-grade chemicals used were purchased from Supelco and Sigma-Aldrich, Saint Quentin Fallavier, France.

Plant Collection

The samples of aerial parts (leaves, stems, tops and fruits) of *Lavandula stoechas* and *Pistacia lentiscus* were collected separately (Figure 1).For this study, we selected two geographical sites in the Tizi-Ouzou region: the mountain and the littoral. For *Lavandulas stoechas* two sites are considered SiteL1 (254m in altitude) and Site L2 (750m in altitude). For *Pistacia lentiscus* two another sites are considered mountain (876m in altitude) and littoral (13m in altitude). According to the period and altitude of the harvest, eight groups of essential oils were obtained for *Pistacia lentiscus*: winter mountain, spring mountain, summer mountain, autumn mountain, winter littoral, spring littoral, summer littoral, and autumn littoral.

Figure 1. Aerial part of *Lavandula stoechas* (a) and *Pistacia lentiscus* (b)

Extraction of Essential Oils

All samples were cleaned of debris and air dried in the dark for 7 days at a temperature of 25–30°C. The essential oils (EOs) were extracted by hydrodistillation process using a Clevenger-type apparatus. Then they were stored in opaque glass bottles at 4 °C until further use.

Extraction of Tannins and Flavonoids from *Lavandula Stoechas*

The tannins and flavonoids are extracted from *Lavandula stoechas* leaves and stems by hydroalcoholic extraction with methanol and water mixture. After filtation of mixture the solvants were evaporated and extract obtained.

GC-MS Analysis of Essential Oils

The chemical analysis of the EOs was carried out by gas chromatography mass spectrometry (GC-MS) using a Trace GC-Ultra instrument coupled to a DSQ-II (singlequadrupole) mass spectrometer supplied by Thermo Fisher Scientific. The chromatographic separation was conducted on a 30 m × 0.25 mm × 0.25 µm TR-5 MS capillary column (5% phenyl and 95% polysiloxane from Thermo-France). One microliter of sample was injected in the splitless mode at 200 °C. The oven temperature was set at 50°C for three minutes; then, it was programmed to increase from 50 to 270°C at a rate of 5/min and then to increase to 330°C at a rate of 10/min. Helium was used as carrier gas (flow rate 1 mL/min). The transfer line was heated to 280°C. Ionization in the electron impact mode was conducted at 220°C with an energy of 70 eV. A full-scan detection for masses (m/z) between 35 and 350 UMA was performed at a rate of 3000 UMA/sec. Each sample of essential oil was diluted 1000 times in hexane containing pentadecane (1/4000 v/v) as the internal standard.

The identification of oil components was assigned by comparison of their retention times and retention indices (RIs). Experimental RIs were calculated as the relative retention times of compounds compared with those of (C10–C40) n-alkanes. Moreover, identification was confirmed by comparing the mass spectra of each molecule with (i) those available in the NIST 05 library of the GC/MS data system and with(ii) the mass spectra published (Adams R.P,2007). The relative quantification of each compound within a sample was based on the peak relative (%) surface measured with the total ion current (TIC) after normalization of the surface area to the internal standard.

Antioxidant Activity of Lentisk Essential Oils

The antioxidant activity of lentisk EOs was assessed using three techniques: the DPPH radical-scavenging test, the ferric reducing antioxidant power (FRAP) assay, and the ABTS radical-cation reduction test. All measurements were spectrophotometry determined and expressed in Trolox equivalent antioxidant capacity (TEAC). Trolox is the hydrophilic equivalent of vitamin E and it was used as the standard for all three tests. All measurements were performed in triplicate and the results are expressed as Trolox equivalent (i.e., mg of Trolox equivalent/g of essential oil; mg TE/g EO) for all tests (i.e., DPPH, FRAP, and ABTS). Vitamin C (ascorbic acid) was tested as the control for the three tests. The results are expressed as mg Trolox equivalent/g of vitaminC.

α-Glucosidase-Inhibitory Activity of Lentisk Extract

The samples of each lentisk organ (stems, leaves and fruits) were dried and then ground using the ball mill.Each extract was obtained by maceration of 200 mg of powder in 1.5 ml of methanol at 70°C for 30 min in an Eppendorf ThermoMixer. The mixture was centrifuged at 1100 G for 5 min. The recovered supernatant was evaporated under a stream of nitrogen. The dry crude extract recovered after evaporation of the methanol was dissolved in a mixture [water (750 µl)/cyclohexane (750 µl)]. After centrifugation, the aqueous phase was collected and lyophilized. The lyophilized aqueous extract was stored at −20°C for LC-MS analysis and evaluation of α-glucosidase activity.The measurement of the effect of lentisk extract on the catalytic activity of α-glucosidase was performed according to the method of (Bachhawat J.A et al. 2011), with a slight modification.

In a 96-well plate, a reaction mixture containing 50 µL of phosphate buffer (50 mM, pH = 6.8), 10µL of α-glucosidase (1 U/mL) and 20 µL of varying concentrations of extract (0, 2, 4, 6, 8 and 10 µg/mL) was preincubated at 37 °C for 15 min. Then, 20 µL of p-nitrophenyl α-D-glucopyranoside (PNPG) (1 mM) was added as a substrate, and the mixture was incubated again at 37 °C for 20 min. The reaction was stopped by adding 50 µL of sodium carbonate (0.1 M). The yellow color produced was read at 405 nm using a plate reader. Acarbose at various concentrations (0.2–1 mg/mL) was included as a positive control. A negative control without extracts was assayed in parallel. The experiment was performed three times for each sample. The UHPLC-ESI-HRMS injected standards were also tested for their effect on α-glucosidase inhibition. The results are expressed as percentages of inhibition, which were calculated according to the following formula :

$$\textbf{Inhibition}\ (\%) = \frac{\left(\mathbf{A_{negatif\ control}} - \mathbf{A_{sample}}\right)}{\mathbf{A_{negatif\ control}}} \times \mathbf{100}\%$$

where $A_{\text{negatif control}}$ corresponds to the absorbance of the control mixture (mixture with the buffer instead of the inhibitor), and A_{sample} represents the absorbance of the samples containing an inhibitor (extracts or acarbose). The α-glucosidase inhibitions of the standards injected for the UHPLC-ESI-HRMS analysis were also tested.

Results and Discussion

Chemical Composition of *Lavandula Stoechas* Essential Oil

The results obtained are summarized in Table 1. These results show that the samples of *Lavandula stoechas* are of the fenchone chemotype which is the majority compound (72 to 77%) of the EO, followed by camphor and cineol. Our results agree with those obtained by Angioni et al. (2006) and Tuttolomondo et al. (2015) for the essential oil of *Lavandula stoechas* collected in Sardaigne and Sicile respectively. On the other hand, our results show that the content of the essential oil in main compounds is influenced essentially by the harvest region but not by the part of the plant used (leaves or flowering tops).

Table 1. Content (in %) of the main compounds of essential oil extracted from leaves of *Lavandula stoechas* harvested on the two sites

Components	Site L1(%)	Site L2(%)
Cineol	02.34	04.19
Fenchone	**77.19**	**72.85**
Camphor	09.34	11.62
Borneol	00.99	01.14
Borneolacetat	03.49	03.13
Mertenylacetat	02.71	03.20
Cubedol	00.71	00.95

Tannin and Flavonoids Yields of *Lavandula Stoechas* Leaves and Tops

The results shown in Table 2 reveal that the highest contents of tannins and flavonoids were obtained with the leaves of *Lavandula stoechas* harvested at the end of May in the Site L1. We recorded a content of 5.7% and 5.8% respectively for flavonoids and tannins.

Table 2. Yield of flavonoids and tannins extracted from leaves and tops of *Lavandula stoechas*

Components	%Site L1	%Site L2
Flavonoids/Leaves	2.96	**5.80**
Tannins/Leaves	3.96	**5.70**
Flavonoids/Tops	1.06	1.60
Tannins/Tops	1.06	3.34

Multivariate Analysis of All the Components Detected in the EOs of *Pistacia Lentiscus*

In order to show differentiation of the chemical compositions between the different EOs, a principal component analysis (PCA) was carried out on the 47 major compounds annotated above (Figure 1). The two axes of the

PCA show 78.6% of the variation. The score plot (Figure 2A) on Axis 1 separates the samples of the two groups according to the sampling site. In each group, the samples for seasons are separated based on Axis 2 of the PCA. Winter and autumn appear mainly separated by Component 2. The mountain group occupies a larger space than the littoral one (i.e., distances between the four seasons were higher for the mountain EOs). This indicates that the variations in the compositions according to the harvesting season were higher at the mountain than at the coastal site. The loading plot (Figure 2B) shows that the most discriminating compounds were the "major" compounds, which appeared at least once at a level greater than 5%.

According to the score and loading plots, the compositions of the EOs analyzed could be subdivided into five sub-chemotypes: Groups 1, 2, 3, 4, and 5. A difference in altitude is usually accompanied by changes in a range of environmental conditions such as temperature, water precipitation, wind exposure, sunlight intensity, UV radiation, and air humidity (Edreva. A et al. 2008). Climatic conditions at (relatively) high altitudes are mainly lower average temperatures, increased thermal amplitude between day and night, and higher light intensity. Such environmental conditions cause plants to change their morphology, physiology, and productivity in order to protect themselves and adapt to such stressful conditions (Edreva. A et al. 2008). In order to evaluate a potential link between our EOs' profiles and their antioxidant properties, further studies were performed with a primary focus on antioxidant activity measurements.

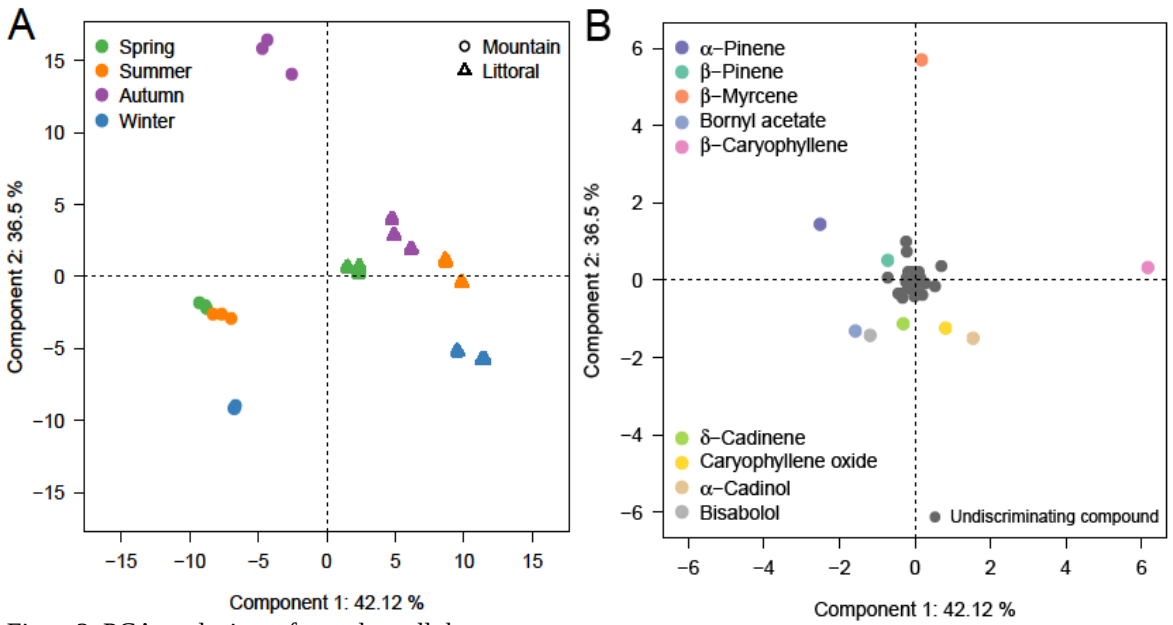

Figure2. PCA analysis performed on all the compounds from the essential oils of Pistacia lentiscus leaf samples collected at the two altitude sites (△ for littoral versus ◯ for mountain) and in 4 consecutive seasons: (A) score plot of PC1 versus PC2 scores; (B) loading plot of PC1- and PC2-contributing EO compounds (the most discriminating compounds are represented in color).

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Antioxidant Activity of EOs of *Pistacia Lentiscus*

The antioxidant activities (AOx) of the different essential oils, assessed by the ferric reducing power (FRAP), free radical scavenging activity (DPPH), and the ABTS radical cation reduction tests are presented in Table 3.

Table 3. Evaluation of the antioxidant activities of Pistacia lentiscus essential oils using FRAP, ABTS, and DPPH tests

Essential oil samples		FRAP (mg TE/gEO)	ABTS (mg TE/g EO)	DPPH (mgTE/g EO)
Mountain	Winter	11.3 ± 0.1	0.28 ± 0.01	0.12 ± 0.0
	Spring	9.6 ± 0.3	0.32 ± 0	0.44 ± 0.01
	Summer	22.3 ± 0.2	0.09 ± 0.01	0.08 ± 0.01
	Autumn	14.3 ± 0.1	0.24 ± 0.01	0.11 ± 0.0
Littoral	Winter	6.4 ± 0.9	0.09 ± 0	0.06 ± 0.0
	Spring	6.6 ± 1.1	0.23 ± 0.01	0.06 ± 0.0
	Summer	15.7 ± 0.5	0.30 ± 0.01	0.08 ± 0.0
	Autumn	14.8 ± 0.4	0.13 ± 0.01	0.06 ± 0.0
Altitude*Season interaction		***	***	***
Vitamin C(mg TE/g vit C)		1083 ± 144	894 ± 88	1348 ± 192

α-Glucosidase Inhibitory Activity

The pairwise comparison through Kruskal-Wallis test adjusted with Bonferroni allowed to study the significance of the variation of this inhibition according to the organ and also the location. The results of this statistical test are shown by the letters (groups, a, b and ab) in Figure 3. Depending on the part of the plant used, effect is significant between stem barks and fruits, and between leaves and fruits.

We also tested acarbose (positive control), a drug (α-glucosidase inhibitor) used for the treatment of type 2 diabetes. The inhibition of acarbose on α-glucosidase is about 100%. Comparing the effect of these extracts with acarbose we can say that the inhibitory effects of acarbose and stem bark extracts on α-glucosidase are comparable

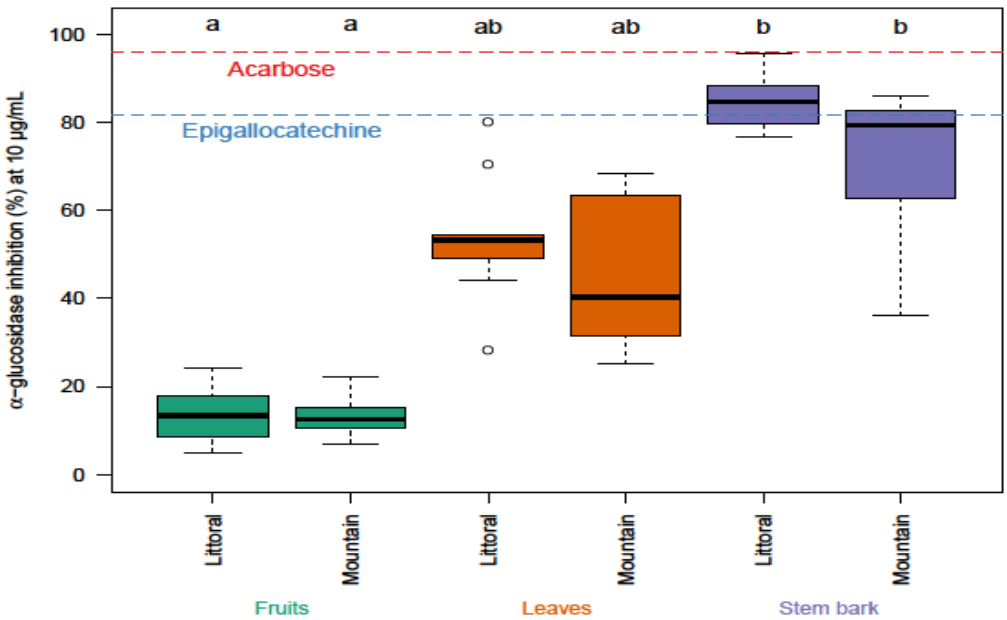

Figure 3. Boxplot showing the inhibitory effect of leaves, stems barks and fruits of lentisk from mountain and littoral on α-glucosidase.

Conclusion

Essential oils of *Lavandula stoechas* are essentially of fenchone chemotype. Essential oils from the leaves of *Pistacia lentiscus* contain mainly 47 compounds. EOs from coastal site were characterized by high amounts of β-caryophyllene. Those from the mountain site were seasonally variable. The chemical class of monoterpene hydrocarbons was higher in the mountains.

The results of this work revealed that the stem bark and leaves from *Pistacia lentiscus* harvested from different areas of Algeria presented a significant activity of α-glucosidase inhibition. The stem barks harvested at the littoral had the highest inhibitory activity toward α-glucosidase. This activity was comparable to that of acarbose. The results collected in this present work show that the species *Lavandula stoechas* and *Pistacia lentiscus* are rich in bioactive molecules with therapeutic interest. This explains their strong use in traditional Algerian medicine.

Recommendations

The goals of the nutrition labeling are to reduce consumer confusion about food labels, help consumers make healthy food choices, and provide an incentive for firms to improve the nutritional quality of food. Nutrition information on food labels should be more understandable to consumers. If necessary, training on reading nutrition labels and evaluating nutrition labels should be provided. The limitation of this study was obtaining the nutrient label of the packaged foods. In this study, only 1000 foods were examined. To obtain more meaningful results, more commercially packaged foods should be studied. In addition, the lack of a previous study on this topic shows the originality of the study, but since there is no source to discuss the results in the discussion section, the study is discussed with its own data.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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